# Microbial Quality of Mangoes from selected markets in Accra, Ghana

Godfred Futagbi [1], Magdalene Addo[1], Precious Agbeko Dzorgbe Mattah[2] and Eric Sampane Donkor[3]

[1.]Department of Animal Biology and Conservation Science, School of Basic and Applied Sciences, University of Ghana, Accra, Ghana
[2.]Directorate of Academic Planning and Quality Assurance, University of Cape Coast, Cape Coast, Ghana
[3.]Department of Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana
gfutagbi@ug.edu.gh

**Abstract:** Studies have shown external contamination and internalization of pathogenic microbes in vegetables and fruits. Contamination of vegetables and fruits with pathogens poses health risk to consumers because some vegetables and most fruits are eaten raw. This study was done to examine contamination of mangoes with microbes. Standard microbiological methods were used to enumerate microbes in mangoes sampled from three markets and based on stage of ripeness and level of scars and punctures. All the mangoes sampled were contaminated both internally and externally with bacteria or yeast, however, 12.5% were not internally contaminated with coliform, faecal coliform, yeast or moulds. Significant differences were observed in coliform and faecal coliform counts of mangoes based on the market they were sampled from ($p < 0.05$). Higher proportions of very ripe and ripe mangoes were contaminated both internally and externally compared to unripe ones ($p < 0.05$). Likewise, higher proportions of mangoes with numerous or moderate scars were contaminated, both externally and internally, compared to mangoes with few scars and it was significant for coliform, faecal coliform and yeast and moulds counts ($p < 0.05$). Significant variations were observed in all the microbial counts of both internal tissues and external surfaces among very ripe, ripe and unripe samples ($p < 0.05$). Significant variations were also seen in all the internal microbial counts and external total, coliform, faecal coliform counts among numerous, moderate and few scars or punctures ($p < 0.05$). However, 25% of the mangoes sampled had internal coliform and faecal coliform counts within acceptable limits. Moreover, no pathogenic *E. coli* were detected in internal tissues and external surfaces of the mangoes. The study shows that mangoes sold in markets in Accra can pose health risk to consumers, particularly very ripe ones and those full of scars or punctures.

## 1. Introduction

Vegetables and fruits are exposed to the soil and may come into contact with animals, including some insects which do not only perch on vegetables and fruits but also insert their ovipositor into them to lay eggs creating punctures and scars. Microorganisms may enter vegetables through such punctures and cuts (Ryall and Pentzer, 1982). Fruits and vegetables are therefore not protected from microbial contamination, even those hanging on trees. Other factors that expose vegetables and fruits to microbial contamination include poor quality irrigation water, poor hygienic conditions during harvest, post-harvest handling and processing, transport and storage (FDA, 1998). These mean that the probability of having fruits and vegetables contaminated is high.

More seriously, outbreaks of human diseases associated with the consumption of food especially, raw fruits and vegetables are frequent occurrence not only in developing countries but also, developed countries (FDA, 1998, Kaferstein, 2003). Studies have

shown external contamination and internalization of pathogenic microbes in some vegetables and fruits (Mensah *et. al*., 2001; Solomon *et al*., 2002; Obeng *et al*., 2007, Donkor *et al*., 2010). These findings show the necessity to evaluate other fruits, especially, ready-to-eat ones.

In Ghana, mango is one of the most important horticultural cash crops for both local and international markets and thought to be the non-traditional export crop to fetch the highest foreign exchange for the country (Banson and Egyir-Yawson, 2014). However, some Ghanaian crops including mangoes have been intercepted and rejected in international markets on similar sanitary and phytosanitary grounds (CTA, 2014).

The objectives of this study were to determine the level of microbial contamination in mango sold in some Ghanaian markets and the possible contamination with pathogenic *E. coli*. This study, therefore, reports microbial load of mango and the

extent of contamination in relation to source, stage of ripeness and frequency of scars or punctures.

## 2. Material and Methods
### 2.1 Study Area and sample collection

Samples were collected randomly from three markets, two in the Accra metropolis and one in Madina, a suburb, from January to April. Kaneshie market in Accra is a modern market, however, with a sprawling chaos of stalls, shops and street vendors. Madina market is found in the La-Nkwantantanag Madina district of Greater Accra region. Just like Kaneshie market, Madina market was overflowing with pavement stalls and street vendors. Mallam Atta market, on the other hand, was the least developed among the three.

According to the vendors, most of the fruits came from large mango farms at Dodowa, also in the Greater Accra Region. Fruits collected were put into aseptic bags and sent immediately to the laboratory for analysis.

Mangoes were sampled based on their state of ripeness, that is, if they were unripe, ripe or very ripe. Unripe, ripe and very ripe mangoes were defined as hard, soft but will not yield to pressure and soft and yield to pressure, respectively. Mangoes were also sampled based on the abundance of scars or punctures. Scars or punctures were few, moderate or numerous if they were less than 3, from 3 to 5 and more than 5 per $10cm^2$, respectively. Common varieties such as Keitt, Kent and Haden were included in the study. In all, forty (40) mangoes were collected and analyzed.

### 2.2 Analysis of the Internal Tissues for microbes

Total Plate Counts (TPC), Coliform Plate Counts (CPC), Faecal Coliform Plate Counts (FCPC) and Yeast, Mould Plate Counts (YMPC) and Pathogenic *E.coli* were enumerated using direct culture methods as described previously (Marshall, 1992). Briefly, Plate Count agar (SPCA), Eosin Methylene Blue agar (EMBA), Violet Red Bile Glucose (VBRGA) were prepared and used in detection of enterobacteria,

Potato Dextrose agar (PDA) and Sorbitol MacConkey (SMA) were used, respectively, to enumerate TPC, CPC, FCPC, YMPC and pathogenic *E. coli*. Mango fruits were disinfected with 70% ethanol at portions where incisions were made.

The fruits were opened up using sterile blades and 100μL of juice from inside the fruits were pipetted using sterile pipette tips. The 100uL fruit juices were added to 900μL of standard saline solution. 10uL of first dilutions of samples were pipetted and transferred into respective dishes and the prepared agars were gently poured into appropriate dishes containing the first dilutions and swirled gently. The set-ups were left on the working slab for the agar to set after which incubation was done for 24 hours to allow growth of microbes. After incubation, the bacteria colonies formed were counted using a colony counter and microbial counts of the mangoes computed.

### 2.3 Analysis of External Surface

This was done to enumerate microbes on the external surfaces of the mangoes. An outer surface area of $1cm^2$ of each mango was swabbed using a cotton swab. The swab was then dipped into 100uL of standard saline solution, from which first and second dilutions were made. The prepared agars were added to the diluted samples. The cultures were incubated and analyzed as described above.

### 2.4 Statistical Analysis

Results from the laboratory analyses were first entered and organized in MS Excel and then analyzed using Graph Prism Statistical software (Prism, GraphPad Software, San Diego, CA, USA). Student's t-test was used to compare the microbial load and z-test the proportions of various categories.

## 3. Results
### 3.1 Proportion of Mangoes Contaminated with Microbes.

All the mangoes sampled were contaminated both internally and externally with bacteria or yeast, however, 12.5% and 11.5% were not internally and externally contaminated, respectively, with coliform, faecal coliform, yeast or moulds. Proportions of mangoes contaminated with CPC and FCPC were significantly lower in mangoes from Mallam Atta compared to samples from Madina and Kaneshie ($p<0.05$).

Proportions of mangoes contaminated were also affected by the stage of ripeness. Higher proportions of very ripe and ripe mangoes were contaminated both internally and externally compared to unripe ones and the proportion internally contaminated with CPC, FCPC and YMPC were significantly lower in unripe mangoes compared to the ripe ones ($p<0.05$). Likewise the proportion of mangoes contaminated was associated with number of scars/punctures. Generally, higher proportions of mangoes with numerous or moderate scars were contaminated, both externally and internally, compared to mangoes with few scars and it was significant for CPC, FCPC and YMPC ($p<0.05$; Table 1).

### 3.2 Microbial Load of Internal Tissues of Fruits Compared between Different Markets.

The geometric means of internal microbial loads of fruits collected at the different markets are presented in Table 2 below. TPC was significantly higher in samples from Madina compared to those from Kaneshie and Mallam Atta ($p<0.05$) but did not differ between Kaneshie and Mallam Atta. Also, CPC was similar for Madina and Kaneshie, however,

significantly lower in samples from Mallam Atta compared to those from Madina and Kaneshie (p<0.05). Though FCPC and YMPC were higher in Madina as compared to Kaneshie and Mallam Atta markets, there was no significant difference among them (p> 0.05). The microbial loads were generally high for the external surfaces of the mangoes but there were no significant differences among the markets with regard to the parameters; TPC, CPC, FCPC and YMPC (p>0.05; Table 2).

Table 1: Proportions of Contaminated Mangoes Based on Source, State of Ripeness and Level of Scars/Punctures for Internal and External Tissues

| Market | N | TPC (%) | | CPC (%) | | FCPC (%) | | YMPC (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Int. | Ext. | Int. | Ext. | Int. | Ext. | Int. | Ext. |
| Madina | 19 | 94.7 | 92.9 | 78.9 | 92.9 | 78.9 | 92.9 | 52.6 | 92.9 |
| Kaneshie | 11 | 90.9 | 100.0 | 90.9 | 100.0 | 72.7 | 100.0 | 72.7 | 90.9 |
| Mallam Atta | 10 | 80.0 | 90.0 | 40.0 | 10.0 | 0.0 | 50.0 | 50.0 | 40.0 |
| Overall | 40 | 90 | 91.4 | 82.5 | 71.4 | 72.5 | 82.9 | 57.5 | 77.1 |
| | | | | | | | | | |
| **Stage of ripeness** | | | | | | | | | |
| Very ripe | 9 | 100.0 | 100.0 | 88.9 | 100.0 | 88.9 | 100.0 | 66.7 | 92.3 |
| Ripe | 15 | 100.0 | 100.0 | 86.7 | 75.0 | 73.3 | 75.0 | 66.7 | 75.0 |
| Unripe | 16 | 75.0 | 78.6 | 37.5 | 42.9 | 25.0 | 71.4 | 37.5 | 64.3 |
| | | | | | | | | | |
| **Scars/punctures** | | | | | | | | | |
| Numerous | 9 | 100.0 | 100.0 | 88.9 | 100.0 | 88.9 | 100.0 | 66.7 | 87.5 |
| Moderate | 19 | 94.5 | 90.0 | 84.2 | 80.0 | 63.2 | 80.0 | 57.9 | 80.0 |
| Few | 12 | 75.0 | 88.7 | 25.0 | 52.9 | 25.0 | 76.5 | 41.7 | 70.6 |

Table 3: Microbial Load of Internal Tissues and External surface of Fruit at Different Stages of Ripeness

| | | TPC | CPC | FCPC | YMPC |
|---|---|---|---|---|---|
| **Internal** | **Ripeness** | ---------------------Mean(±SEM) cfu mL$^{-1}$---------------------- | | | |
| | Unripe | $5.8\times10^2$ $(1.7\times10^2)$ | $1.6\times10^2$ $(8.7\times10^1)$ | $8.8\times10^1$ $(5.2\times10^1)$ | $1.7\times10^2$ $(8.8\times10^1)$ |
| | Ripe | $3.6\times10^3$ $(6.9\times10^2)$ | $1.4\times10^3$ $(3.8\times10^2)$ | $6.9\times10^2$ $(1.5\times10^2)$ | $3.87\times10^2$ $(9.7\times10^1)$ |
| | Very Ripe | $7.3\times10^3$ $(1.6\times10^3)$ | $5.0\times10^3$ $(1.7\times10^3)$ | $3.2\times10^3$ $(1.0\times10^3)$ | $2.3\times10^3$ $(9.21\times10^2)$ |
| | | ----------------------Mean(±SEM) cfu cm$^{-2}$---------------------- | | | |
| **External** | Unripe | $2.1\times10^3$ $(1.0\times10^3)$ | $1.3\times10^3$ $(3.4\times10^2)$ | $9.1\times10^2$ $(5.6\times10^2)$ | $7.6\times10^3$ $(3.8\times10^3)$ |
| | Ripe | $3.0\times10^3$ $(7.0\times10^2)$ | $1.7\times10^3$ $(3.6\times10^2)$ | $1.3\times10^3$ $(3.3\times10^2)$ | $9.0\times10^2$ $(1.7\times10^2)$ |
| | Very Ripe | $2.0\times10^4$ $(5.3\times10^3)$ | $9.9\times10^3$ $(1.9\times10^3)$ | $7.5\times10^3$ $(1.6\times10^3)$ | $3.7\times10^3$ $(1.2\times10^3)$ |

**3.3 Microbial Load of Internal Tissues and External surface Compared among Different Stages of Fruit Ripeness**

Significant variations were observed in all the internal microbial counts among very ripe, ripe and unripe samples (p<0.05) with very ripe having the highest counts and unripe the lowest, except YMPC, which though higher in ripe compared to unripe mangoes, was not significant (Table 3). Significant variations were also observed in all the external microbial counts among very ripe, ripe and unripe samples (p<0.05) with very ripe having the highest counts and unripe the lowest (Table 3).

**3.4 Microbial Load of Internal Tissues of Mango Compared Between Different States of Damage**

Significant variations were also seen in all the internal microbial counts; TPC, CPC, FCPC and YMPC among numerous, moderate and few scars/punctures (p<0.05), with numerous scars/punctures having the highest counts and few scars/punctures the lowest (Table 4). Significant disparities were also seen in all the external microbial counts; TPC, CPC and FCPC among numerous, moderate and few scars/punctures (p<0.05), with numerous scars/punctures having the highest counts and few scars/punctures the lowest. With regard to

YMPC, numerous and moderate scars/punctures were significantly higher than few scars (p<0.05) but there was no significant difference between numerous and moderate scars/punctures (p>0.05; Table 3).

Table 2: Microbial Load of Internal and External Tissues of Fruits collected from Different Markets

| | | | TPC | CPC | FCPC | YMPC |
|---|---|---|---|---|---|---|
| **Internal** | **Market** | | --------------------**Mean(±SEM) cfu mL$^{-1}$**-------------------- | | | |
| | Madina | | $4.8\times10^3$ $(1.0\times10^3)$ | $2.5\times10^3$ $(9.7\times10^2)$ | $1.4\times10^3$ $(5.4\times10^2)$ | $9.9\times10^2$ $(3.8\times10^2)$ |
| | Kaneshie | | $1.8\times10^3$ $(4.7\times10^2)$ | $1.3\times10^3$ $(3.1\times10^2)$ | $1.2\times10^3$ $(4.7\times10^2)$ | $4.3\times10^2$ $(1.5\times10^2)$ |
| | Mallam Atta | | $1.8\times10^3$ $(1.1\times10^3)$ | $7.7\times10^2$ $(6.0\times10^2)$ | $0.00$ | $1.8\times10^2$ $(1.0\times10^2)$ |
| | | | --------------------**Mean(±SEM) cfu cm$^{-2}$**---------------------- | | | |
| **External** | Madina | | $8.9\times10^3$ $(1.8\times10^3)$ | $4.4\times10^3$ $(1.4\times10^3)$ | $1.6\times10^3$ $(6.4\times10^2)$ | $3.4\times10^3$ $(1.5\times10^3)$ |
| | Kaneshie | | $4.6\times10^3$ $(2.1\times10^3)$ | $3.3\times10^3$ $(2.6\times10^3)$ | $2.3\times10^3$ $(1.1\times10^3)$ | $8.5\times10^3$ $(4.4\times10^3)$ |
| | Mallam Atta | | $6.6\times10^3$ $(5.4\times10^3)$ | $2.4\times10^3$ $(1.8\times10^3)$ | $1.4\times10^3$ $(1.3\times10^3)$ | $2.0\times10^3$ $(1.3\times10^3)$ |

Table 4: Microbial Load of Internal Tissues of the Fruit in Different States

| | | TPC | CPC | FCPC | YMPC |
|---|---|---|---|---|---|
| Internal | **Scars/Punctures** | --------------------**Mean(±SEM) cfu uL$^{-1}$**------------------------ | | | |
| | Few | $4.8\times10^2$ $(1.5\times10^2)$ | $1.5\times10^2$ $1.2\times10^2)$ | $1.0\times10^2$ $(6.7\times10^1)$ | $1.3\times10^2$ $(8.2\times10^1)$ |
| | Moderate | $3.1\times10^3$ $(6.1\times10^2)$ | $1.2\times10^3$ $(3.2\times10^2)$ | $5.6\times10^2$ $(1.3\times10^2)$ | $3.6\times10^2$ $(9.2\times10^1)$ |
| | Numerous | $7.4\times10^3$ $(1.7\times10^3)$ | $5.1\times10^3$ $(1.8\times10^3)$ | $3.2\times10^3$ $(1.0\times10^3)$ | $2.4\times10^3$ $(9.3\times10^2)$ |
| | | --------------------**Mean(±SEM) cfu cm$^{-2}$**------------------------ | | | |
| External | Few | $7.8\times10^2$ $(2.3\times10^2)$ | $7.4\times10^2$ $(2.8\times10^2)$ | $7.0\times10^2$ $(6.0\times10^2)$ | $1.2\times10^3$ $(1.1\times10^3)$ |
| | Moderate | $3.6\times10^3$ $(9.1\times10^2)$ | $3.2\times10^3$ $(2.8\times10^3)$ | $1.7\times10^3$ $(4.6\times10^2)$ | $5.8\times10^3$ $(2.9\times10^3)$ |
| | Numerous | $2.1\times10^4$ $(5.4\times10^3)$ | $9.8\times10^3$ $(1.9\times10^3)$ | $7.6\times10^3$ $(1.7\times10^3)$ | $3.8\times10^3$ $(1.3\times10^3)$ |

## 3.5 Microbial limits and Pathogenic *E.coli* contamination

The mean TPC ($<10^5$) and YM ($<10^4$) of the internal tissues and external surfaces were lower than acceptable limits. However, the mean CPC and FCPC were far higher than acceptable counts. Twenty-five percent (25%) of the mangoes sampled had internal microbial counts within acceptable CPC and FCPC limits (<100 cfu/g and 0 cfu/g, respectively; NRC, 1985). Also, 56.3% of mangoes with few scars/punctures or were unripe had acceptable CPC and FCPC counts. No pathogenic Pathogenic *E. coli* were detected in internal tissues and external surfaces of the mangoes.

## 4. Discussions

Very high proportions of mangoes were internally and externally contaminated with microbes but Mallam Atta market had the lowest. This seems to suggest that the contamination might have occurred at the point of retail rather than at the farm gates. The proportions of fruits contaminated internally or externally was influenced by the stage of ripeness and level of scars or punctures with very ripe and numerous scars/punctures having the highest. This is expected because it has been shown that the frequency of internalization of microbes in mango is higher for ripe mangoes compared to immature ones (Penteado *et al.,* 2007). Interestingly, all the very ripe fruits had numerous scars or punctures. However, it is not

surprising because, though immature fruits are also attacked by fruit flies, ripe fruits are preferred for ovipositioning (Liquido *et al.*, 1989; Kimbokota, 2013) and the structure of ripe fruits are more delicate than unripe ones and can easily be damaged through handling, transportation and storage, among others. Damages to the natural structure such as punctures, wounds, cuts and splits on fruits and vegetables are known to facilitate microbial entry (Ryall and Pentzer, 1982). Microorganisms may be trapped by the exudations on the fruits caused by the damages. This may account for the high proportion of fruits with numerous scars or punctures having external surfaces contaminated. The public health importance of the high frequencies of contamination depends on the species and the microbial load.

Whereas total plate counts and level of yeast and moulds were lower than the minimum acceptable limits for all the mangoes analyzed, only 25% of the mangoes sampled had internal coliform and faecal coliform counts within acceptable limits (<100 and 0, respectively; NRC, 1985). The presence of faecal coliform is an important indicator of faecal contamination and poor food hygiene (WHO, 1993, Edberg *et al.*, 2000). This means that large proportion of mangoes on the markets do not meet internationally acceptable standards and are not wholesome for consumption. This also suggests mangoes that are sold in these markets can be sources of infection. However, about (56.3%) of unripe mangoes or those with few scars/punctures had acceptable coliform and faecal coliform counts. This implies that unripe mangoes in the markets are relatively safe for human consumption compared to ripe ones. Human and animal wastes used as fertilizers or irrigation water contaminated by faecal matter are reported sources of contamination of fresh vegetables and fruits with microbes (ICMSF, 1986, Donkor *et al.*, 2010) but it is not clear if the mangoes were exposed to faecal contaminants on the farms. The sources of microbial contaminants of mangoes require further investigations.

In conclusion, the study has shown both external and internal contamination of mangoes displayed for sale on the markets and the level of contamination increased with the number of scars or punctures on the mangoes. It is advisable, therefore, that consumers avoid purchasing mangoes with scars and punctures. It is recommended that further studies are done to ascertain the sources of microbial contaminants.

**Acknowledgements:**

**Corresponding Author:**
Dr. Godfred Futagbi
Department of Animal Biology and Conservation Science, School of Basic and Applied Sciences, University of Ghana, Accra, Ghana
E-mail: gfutagbi@ug.edu.gh

**References**
1. Banson KE, Egyir-Yawson A. Socio-economic impact of fruit flies control in mango production in Ghana, evidence from "Manya Krobo". J Agr Sci Tech 2014;4(6):454-463.
2. CTA. Increased interceptions of 'harmful organisms' overhang some ACP horticultural exports to the EU. Agritrade Informed Analysis, Expert Opinions. Technical Centre for Agricultural and Rural Cooperation (ACP-EU) 2014; Available at: http://agritrade.cta.int/en/layout/set/print/Agriculture/Topics/SPS-Food-safety/Increased-interceptions-of-harmful-organisms-overhang-some-ACP-horticultural-exports-to-the-EU.
3. Donkor ES, Lanyo R, Kayang BB, Quaye J, Edoh DA. Internalization of Microbes in Vegetables: Microbial Load of Ghanaian Vegetables and the Relationship with Different Water Sources of Irrigation. Pak J Biol Sci 2010;13(17): 857-861.
4. Food and Drug Administation (FDA). Guide to minimize microbial food safety hazards for fresh fruits and vegetables 1998; Available at http:///www.cfsan.fda.gov/~dms/ prodguid.html.
5. NRC. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academies Press, Washington, D.C. 1985.
6. ICMSF. The ICMSF sampling plans and recommended microbiological limits for vegetables, fruits and nuts. In: Microorganisms in Foods 2, Sampling for Microbial Analysis: Principles and Specific Applications, 2nd ed. Palo Alto, ICMSF Blackwell Scientific Publications CA 1986;197–202.
7. Kaferstein F. Foodborne disease in developing countries: Aetiology, epidemiology and strategies for prevention. Int J Environ Res 2003:13(Suppl 1):S161-8.
8. Kimbokota F, Njagi PGN, Torto B, Ekesi, S and Hassanali A. Responses of *Bactrocera invadens* (Diptera: Tephritidae) to volatile emissions of fruits from three hosts. J Biol Agric Healthcare 2013;3:53–60.
9. Liquido NJ, Cunningham RT, Couey HM. Infestation rates of papayas by fruit flies (Diptera: Tephritidae) in relation to the degree of fruit ripeness. J Econ Entomol 1989;82, 213-219.

10. Mensah P, Armar- Klemesu M, Hammond AS, Haruna A, Nyaku R. Bacteria contaminants of lettuce, tomatoes, beef and goat meat from the Accra Metropolis. Ghana Med J 2001;35: 1-6

11. Obeng AS, Kwakye-Nuako AG, Asmah RH and Ayeh-Kumi PF. Pathogenic parasitic microbes associated with fresh vegetables consumed in Accra. GJAHS 2007; 1: 11-15.

12. Penteado AL, Eblen BS, Miller AJ. Evidence of *Salmonella* internalization into fresh mangoes during simulated postharvest insect disinfestations procedures. *J Food Prot* 2007; 67: 181-184.

13. Ryall AL, Pentzer WT. Handling Transportation and Storage of Fruit and Vegetables AVI. Publishing Co. Inc., Connecticut, 1982: 610.

14. WHO, Guidelines for Drinking Water Quality 1993; 2$^{nd}$ ed., WHO, Geneva.

15. Edberg SC, Rice EW, Karlin MJ, Allen J. *Escherichia coli*: The best biological drinking water indicator for public health protection. J Applied Microbiol 1999;88: 106S-116S.

2/28/2016