Microbiological Profile of Milled Tomatoes in Madina Market in the Greater Accra Region of Ghana

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Abstract
The microbiological safety of milled tomatoes in the market of Madina in the Greater Accra Region of Ghana was assessed. Samples were taken before and after milling in triplicates, analyzed for microbiological safety in the laboratory and tested for aerobic mesophiles, yeasts and molds, coliforms, E. coli and Staphylococcus aureus. Counts for aerobic mesophiles generally decreased after milling. Yeasts and molds populations decreased from $6.0 \times 10^8$ before milling to $8.0 \times 10^4$ cfu/g after milling. Test for Staphylococcus aureus and E. coli were negative. Swabs from milling accessories such as pestles, knives, bowls and machine funnels tested positive for coliforms and exceeded threshold level of $1 \times 10^4$ units ($5.96 \times 10^4$ – $6.00 \times 10^9$ cfu/g), aerobic mesophiles ($2.00 \times 10^3$ – $1.97 \times 10^{13}$ cfu/g) and yeasts and molds ($2.20 \times 10^2$ – $1.17 \times 10^7$ cfu/g). There is therefore the need for food safety education and enforcement of safety regulations such as good manufacturing practices (GMPs) by the Food and Drugs Authority of Ghana.

INTRODUCTION

Tomato fruit are consumed in many forms and used for many dishes in Ghana and the world at large. According to statistics, tomatoes are an extensively grown commodity, with production figures in 2005 approximately reaching 35 million tonnes in developed countries and 90 million tonnes in developing countries [1]. In 2011, production figures reached about 160 million tonnes, about 15% of total vegetable production. Tomatoes being one of the most grown vegetables in the world, it is envisaged that production would be higher in 2014 than reported for preceding years [2]. Similarly, tomato production in Ghana over the past 7 years increased from 180,000 tonnes in 2007 to 320,000 tonnes in 2012 (Figure 1).

Processing of tomato fruit can contribute to food poisoning when not properly done [3,4]. There are mills in most of the local markets in Ghana used for processing tomatoes and other food commodities. These mills, owned by corporate groups and individuals, are operated on commercial basis and are patronized mainly by food vendors who prepare food for sale to the public. Milling tomatoes is one such common activity in Ghana and the tomatoes are often milled, mixed with onions or ginger and used to prepare stews and sauces for rice and kenkey among other Ghanaian dishes. Figure 2 depicts how tomato fruits are handled and milled. Sometimes, the milled tomatoes are not cooked before consumption but eaten fresh when mixed with chili.

Cutting, slicing, chopping, milling, and mixing are important processing steps to which fresh fruits and vegetables are commonly exposed [3,5]. Processing operations that damage fruits and vegetable tissues can lead to increased microbial populations [6]. Poorly sanitized equipment may harbor contaminants that are transferred to the fruits and vegetables during processing.

For example, *Geotrichum candidum*, sometimes called machinery mold or dairy mold, can accumulate on processing equipment and contaminate fruit and vegetables upon contact [7]. Milling processes may also cause vegetable tissue fluids to express onto outer surfaces of vegetable and processing equipment. These fluids can serve as nutrients for growth of microorganisms, allowing them to accumulate to higher populations [8].

Milling under unsanitary conditions is of public health concern. Some studies conducted on street foods in Ghana revealed the unsanitary conditions characterizing the sector and mention was made of foods contaminated with bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus* [9,10,11]. Another study considered street food vending in urban Ghana a major cause of health hazards such as foodborne diseases [12]. Bacterial presence in foods in Accra was also recorded by another study on street foods [13]. Polluted waste water for irrigation and material for constructing processing equipment were also mentioned to significantly influence microbial contamination of foods [14,15]. However, no studies were found to target the microbiological safety of milled vegetables used by street food vendors in Ghana, especially those consumed fresh like tomatoes. This study therefore seeks to assess the microbiological safety of milled tomatoes produced in the Madina market in Accra, Ghana. It also sought to identify the sources of contamination and hygiene challenges at milling sites and make appropriate recommendations.

Some microorganisms considered in this work are *Escherichia coli* and *Staphylococcus aureus*, important microorganisms to consider for safety of food products [7,16,17]. Common symptoms of microbiological contamination and food poisoning are nausea, abdominal cramps, headaches and diarrhea. Pregnant women, children and the elderly have been identified by research as the most vulnerable group susceptible to food poisoning and its complications [18]. This may be due to teratogenicity of contaminants, immunodeficiency among the elderly and children [18].

**MATERIALS AND METHODS**

**Sampling**

Samples were collected from milling centers from two sites at the Madina market in Accra. Tomato fruits brought to the market for milling, some of which were mixed with onions and/or ginger, were sampled (about four fruits per sample). Swabs from all four fruits of each sample unit were taken before milling. During the milling process, small amounts of the milled products for each sample were collected at regular intervals in triplicates into sterile stomacher bags. Thus for each sample, three replicates were taken. Those mixed with other ingredients were labeled appropriately. Swabs were also taken from pestles, bowls, benches and knives used and from the funnels of milling machine to ascertain the degree of contamination. Samples were stored on ice to preserve them for immediate laboratory analyses. It was
difficult to take swabs of the interior of milling machines because such a procedure was time consuming and as well, disrupted commercial services rendered.

**pH Determination**

The pH values for fresh tomato samples were determined by poking the electrode of the pH meter (Model 350, Jenway, England) into the fruit. For those mixed with onions or ginger, they were crashed in sterile stomacher bags and their juice used for pH determination. This was to ensure that pH reading represents the whole mixture rather than only tomato fruit. Milled samples were also tested by dipping electrode into pastes. pH values were determined before and after milling tomato fruits samples.

**Microbiological analysis**

Samples were immediately analyzed in the laboratory for their microbial quality by the following procedures:

**Serial Dilution and Enumeration of Microorganisms**

These procedures followed those of [19] with little modifications. For the $10^{-1}$ dilution factor, 10g of each tomato sample was weighed and 90ml of Salt Peptone Solution (SPS) added. Subsequent dilutions were made from $10^{-1}$ to $10^{-9}$, depending on the test method. The Petri dishes were inoculated with 1ml inoculums of the various sample dilutions and poured with about 15 to 20ml of media. PCA medium was poured into plates for aerobic plate counts and were incubated at 30°C for 3 days. OGYEA medium was poured into plates meant for yeasts and molds followed by Chloramphenicol, which acts as an antibacterial agent was added to it. The set plates were incubated at 25°C for five days.

Plates for facultative anaerobes were first poured with TSA and allowed to set after 1 hour and overlaid with VRBA and allowed to set. The set plates were incubated at 37°C and 44°C for different microorganisms.
coli forms and *E. coli* respectively. Plates were removed from the incubators after their respective incubation periods. Good plates (plates with counts between 25 and 250) were selected for each test and colony forming units (cfu) counted using Quebec colony counter.

Baird Parker (BP) medium, plates were inoculated with 0.1ml inoculums of each sample (10⁻¹ dilution) and spread. The formation of typical jet-black colonies with clear zones were expected to ascertain the presence of presumptive *Staphylococcus aureus*.

Confirmatory Tests for microorganisms

**Yeasts and Molds:** Yeasts and molds were confirmed microscopically on typical colonies on OGYEA plates. Sterile cover slips were used to pick single colonies and to spread them on a droplet of sterile distilled water to form a thin smear.

**Escherichia coli:** Good plates for presumptive *E. coli* were selected and 5 typical colonies from each plate were transferred aseptically into the EC Broth in test tubes. Gas collection in Durham’s and cloudiness were expected. 0.1ml of broth from each positive presumptive *E. coli* tube was transferred into the tubes. Two drops of Indole reagent was then added to the contents of each tube. An instant red ring formation on the surface of media in tubes was expected to confirm the presence of *E. coli* in sample.

**Staphylococcus aureus:** 0.1 ml of a 10⁻¹ dilution of sample was spread on Baird-Parker medium in plates. The formation of shiny, jet-black colonies surrounded by clear zones was expected to establish the presence of Staph. aureus.

Calculation for the number of microorganisms in 1g of sample (Coliforms) is given by:

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\text{Number of microbes} = \left( \frac{\text{Number of positive BGBB tubes} \times \text{total number of tubes}}{\text{plate count}} \right) \times \text{(dilution factor)}.
\]

**RESULTS AND DISCUSSION**

**Microbiological Analysis**

**pH of Samples:** Onions and ginger are known to be alkalizing foods or foods that increase the pH of products they are mixed with. In this case, for sample 1, the pH reduced after milling from 4.33 to 4.30 as shown in Table 1. It was expected that pH would rise because of the onions added to the tomatoes but this was insignificant. Increases in pH values of samples 2 and 3 were observed after milling. This could be explained by the fact that sample 2 had a greater percentage of onions in the tomatoes than sample 1. For sample 3, the increased pH could be due to the added ginger.

**Population of aerobic mesophiles in samples:** Plate count results as shown in Table 2 for samples 2 and 3 recorded decreases in the number of microorganisms after milling. However, for sample 1, there was an increase in the number of microorganisms after milling. This inferred that the sample might have been contaminated during the milling process. According to observation, the milling machine had been idle for sometime before sample 1 was milled and collected. This might support the growth and build-up of microorganisms in the interior of the equipment hence the increase in aerobic mesophile counts after milling.

**Population of yeasts and molds in samples:** Table 3 shows a general decrease in population of yeasts and moulds in samples after milling. This inferred that the milling process had been destructive to the yeasts and molds present in the initially unprocessed samples. The reduction in counts obtained after milling can be best justified by the increased temperature normally between 40-50°C during the milling process which is not conducive for the growth of yeasts and molds.

**E. coli:** Confirmatory test showed no counts for *E. coli* in samples.

**Staphylococcus aureus:** There were no positive confirmations for the presence of *Staphylococcus aureus* in the tomato samples as shown in Table 6. There were growths of shiny jet-black colonies on BP plates but without clear zones to establish the presence of *Staphylococcus aureus*. Other species of *Staphylococcus* might be present but not *Staph aureus*. pH values of samples between 4.30 and 4.71 were also not suitable for the growth of *Staph aureus*. As stated in literature, optimal pH for growth ranges between 6 and 7 [7].

**General Hygiene at Milling Sites as Observed**

**Mishandling of Accessories:** It was observed that the mill operators failed to observe basic hygienic principles during the processing and handling of the milled tomato products. They had dirty milling bowls, dirty hands, dirty water containers and knives. The external parts of the equipment were very dirty. The pestles they used were dirty and placed anywhere, not considering the sanitary condition of contact surfaces (Figure 3). To substantiate the effects of improper handling of milling accessories, Table 4 also shows results for microbial counts of aerobic mesophiles, yeasts and molds on pestles, knives, benches, milling bowls and machine funnels.

According to the Food and Drugs Authority (FDA) recommendations, levels of coliform (MPN/g), *S. aureus* (cfu/g), Yeasts & Molds (cfu/g) and *E. coli* (MPN/g) in fruits and vegetables that are ready for consumption should not exceed 1 x 10⁴ units [20]. Unfortunately, tests for coliforms recorded results higher than the threshold level of 10⁴ MPN/g for Yeasts and Molds, counts on pestles and milling bowls were the only that exceeded the threshold whereas those for the working benches, knives and machine funnels were below the level.

**Improper washing and exposure of tomatoes before milling:** Most of the owners of the tomatoes brought to the market for milling were not washing and cleaning the tomatoes well before milling. They washed the tomatoes just once in water. The washing water was not changed. Most of the tomatoes brought for milling were damaged and appeared to have undergone some extent of physical degradation. Some of the tomatoes appeared to have rotten as they appeared moldy. Some had wholesome tomatoes but mixed them with rotten ones. Tomatoes were also left exposed to the environment and flies (Figure 4).

**Improper Waste Disposal:** Wastes were not properly disposed from the operation site. The wastewater after washing of tomatoes was not thrown away but used to clean the...
equipment and other surfaces. Some of the operators used the waste water to mill the tomatoes and for intermittent rinsing of the equipment. It was also realized from interrogation that the processing equipment used for milling tomatoes were not washed with detergents. The operators only use water to rinse the interior of the equipment. Milling surroundings were also not tidy. Waste tomatoes and onions were heaped in baskets for about 2 to 3 days without appropriate handling and disposal. The movement of flies to and from refuse to milling equipment and fruits could possibly have enhanced cross contamination.

**Rust Development in Mills:** The processing equipment was made from mild steel and cast iron instead of stainless steel. For this reason, the equipments were rusty and unsafe for processing food. This condition may expose consumers to metal contaminants in milled products.

All these unhygienic conditions could be the reasons why higher microbial counts were generally observed after milling the tomatoes and could result in foodborne illness [21]. There are guidelines provided by the Codex Alimentarius Commission (CAC) for safe handling of fruit and vegetables which could be followed to reduce food risks [22].

**CONCLUSION**

Tomatoes brought for milling were generally not of good quality as they appeared to have undergone some extent of physical deterioration and were slightly moldy. The tomatoes were also left exposed to flies before milling. Washing of tomatoes was also poor.

Processing line sanitation was not satisfactory, in that no detergents were used in cleaning the processing equipment. Funnels of mills were also left open to dust and fly. Accessories used during processing such as washing bowls, pestles, knives and water containers were very dirty.

The milled tomatoes generally had high counts for aerobic mesophiles and yeasts and moulds. However, there were no counts for *E. coli* and *Staphylococcus aureus* in milled tomato samples. Milling accessories such as knives, benches and bowls also had considerably high microbial counts for aerobic mesophiles and coliforms, revealing the need for good manufacturing practices (GMPs) in place. When milled products are exposed to such contaminants, it poses health risks to consumers and the existence of such threats at high levels does not enhance sustainable development and business environment in the food industry and in the country at large.

This study recommends that thorough education on food hygiene and sanitation be made part of the curricula of public schools to help inform the Ghanaian populace on basic food safety principles. Routine conformity checks done by the FDA of schools to help inform the Ghanaian populace on basic food hygiene and sanitation be made part of the curricula of public schools. The operators should be educated on food hygiene and be made aware of the consequences of not following proper food hygiene procedures.

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