

Occurrence of foodborne bacteria with outbreak potentials and fungi associated with tomato (*Solanum lycopersicum* L.) in postharvest

Abstract

The high-water content of tomato predisposes it to spoilage by bacteria and fungi that can pose significant health threats to consumers. However, parameters such as environment and human factors influence the rate of contamination and microbial species involved. Hence, the present study aims to determine the various pathogenic bacteria and fungi associated with tomatoes sold in some markets in southern Benin. A total of 70 tomatoes were sampled, out of which 35 undamaged tomatoes and 35 spoiled tomatoes were processed for examination of bacterial and fungi contamination by standard culture technique following standard protocols. Results indicated that out of the 70 tomatoes samples analyzed, 85.71% are contaminated with enteric bacteria. *Escherichia coli* was the commonest bacterial contaminant. Foodborne bacteria with outbreak potentials were also detected in analyzed samples. Fungi isolated belong to genera of *Aspergillus*, *Fusarium* and *Mucor*. The most identified species are *Aspergillus ochraceus* (98.91%), *Fusarium oxysporum* (84.28%), *Aspergillus niger* (72.85%) and *Aspergillus versicolor* (35.71%). The presence of these foodborne pathogens raises concern on public health risks associated with the consumption of fresh tomatoes. Efforts should be made to advise farmers to avoid the use of contaminated wastewater for irrigation during cultivation, and discourage purchasing spoiled tomatoes.

Keywords: Tomato, contamination, bacteria, fungi, public health, Benin.

Introduction

“According to World Health Organization (WHO), fruits and vegetables play important role in maintenance of health and prevention of disease” [1]. “Then, the intake of a minimum of 400 g of fruits and vegetables per day for the prevention of chronic diseases and alleviation of several micronutrient deficiencies is recommended” [2]. “However, unhygienically prepared and consumed fruits and vegetables could bring potential risk of acquiring various infectious diseases” [1]. Tomato (*Solanum lycopersicum* L.) is the second most important fruit or vegetable in the world [3], with various health-promoting compounds of tomato such as vitamins, carotenoids and phenolic compounds which have a wide range of physiological properties (anti-inflammatory, antimicrobial, cardio-protective and antioxidant), explained its

nutritional importance [4, 5]. According to Krauss et al. [6], “in addition to consuming the fresh tomatoes fruits, consumers are also used tomatoes in processed products such as soups, juices, and sauces”. “Tomatoes are also classified as climacteric fruits, which exhibited a peak of ethylene production at the start of ripening” [7]. Indeed, fruits ripening occurs when specific enzymes like pectinases and amylases break down pectin and starches and softens and sweetens fruits. However, there is another important factor in the fruit ripening process. This is ethylene, a natural gas that triggers and promotes fruit ripening. Then, climacteric fruits are those that can ripen after being picked, because they are able to produce a lot of ethylene through autocatalytic processes, which lead to the transformation of the hard, unpalatable green tomato into an attractive, brightly colored, succulent and nutritious fruit [5]. “This possibility of climacteric fruits to be picked before ripening, leads producers to pick tomatoes before ripening in field, and store them in multi-product storage places, which leads to cross-contamination of microbial and contributes to the rapid deterioration of tomatoes. Moreover, several factors such as the use of insufficiently treated wastewater for irrigation, the contamination of soil with animal wastes, the application of improperly composted manures to soil and poor or inappropriate hygienic practice during transport contribute in tomato fruits contaminations” [8]. According to Kusumaningrum et al. [9] and Eni et al. [10], “enteric bacteria continue to be major global health problems and are the leading causes of morbidity and mortality”. Unfortunately, in the current context of evolving antibiotic resistance, the resurgence of foodborne diseases poses a great risk to global health [11]. In this context, “control infectious diseases, it is not enough to depend only on chemotherapeutic intervention, but need effort to reduce or eliminate the potential sources of infection” [1]. Then, the present study aims to investigate the risks of microbial contamination associated with tomato fruits sold in southern Benin. This study could contribute to allow the planning and execution of interventions aimed at preventing, reducing or eliminating the contamination of fresh products by pathogenic microorganisms.

Material and methods

Zone of study

The study was carried out in Benin, a country located between the meridians 0°40' and 3°45' East longitude and the parallels 06°15 and 12°25' North latitude [12]. The study sites are local markets of *Adjahonmè*, *Tchikpé*, *Lanta*, *Klouékanmey*, *Hondjin*, *Djotto* and *Ayahohouè*,

located in the municipalities of Klouekanmey, from the department of *Couffo* (Southern Benin).

Sample collection

Samples of tomato fruits of local variety name ‘*Akikon*’ were purchased in the main markets of seven localities of municipalities of Klouekanmey which is one the major production areas of tomatoes in southern Benin. In each market, five different sellers were randomly selected and about 250 gm of two batches of tomato samples (undamaged and spoiled) were purchased. A total of 70 samples were then collected, separately packaged into sterile plastic bags, labelled, and transported to the laboratory for microbiological analyses.

Microbiological analysis

For microbiological analysis, 25 g of each sample and 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on MacConkey agar, Tryptone bile glucuronate (TBX) agar, Xylose Lysine Desoxycholate (XLD) agar and nutrient agar. Plates were incubated at 37 °C for 24 h for colony formation. Distinctive morphological properties of each pure culture were examined based on the shape, colour, border, texture, general appearance of individual bacterial colonies on each plate, and bacteria were isolated and identified on the basis of morphological, cultural and different biochemical tests using API 20E gallery, following standard protocol [13]. “For fungal isolation and identification, 1 ml volume of decimal dilutions were separately placed in Petri dishes, over which, 10 to 15 ml of Sabouraud agar amended with 60 µg/ml chloramphenicol was poured. The plates were incubated at 28 ± 2°C for 7 days. Fungal isolates were sub-cultured on malt extract agar (MEA), and identification was carried out by using a taxonomic scheme primarily based on morphological characters, using the methods described” by Singh et al. [14].

Statistical analysis

Experiments were performed in triplicate, and data analyzed were mean subjected to one-way ANOVA. Means were separated by the Tukey’s multiple range test when ANOVA was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

Results and discussion

Results obtained during the evaluation of bacterial contamination of tomato samples collected in the municipality of *Klouekanmey* (Southern Benin) are presented in Tables 1 and 2. On

one hand, results obtained show that the level of tomato samples contamination depends on the collection area (Table 1). This result could be explained by cultural practices such as the use of insufficiently treated wastewater for irrigation, the contamination of soil with animal wastes, the application of improperly composted manures to soil and the poor or inappropriate hygienic practice during transport [8]. It could also be explained by the environmental conditions as reported by Son et al. [15]. On the other hand, results indicated that the level of the contamination also depends on the grade (undamaged or spoiled) of analyzed tomatoes (Table 2). Indeed, taking into account of obtained results, spoiled tomato samples analyzed are more contaminated with pathogenic bacteria than undamaged tomato samples analyzed. These results could be explained by the fact that fresh fruits, including tomatoes, have a natural protective barrier (skin) that acts effectively against most plant damage and pathogenic microorganisms. However, during their growth in fields or harvesting, this protection can be accidentally removed and the fruits can be contaminated with microorganisms [16]. The high-water activity (A_w) of tomatoes promotes the rapid growth of microorganisms and explains their rapid deterioration after contamination. Moreover, the advanced maturity state of tomatoes could also increase their susceptibility to being attacked by parasites and microorganisms. Indeed, the fruits are mechanically protected by the pectins which constitute a "glue" between cells, and gives them rigidity. However, when tomato fruits ripen, endogenous pectinases begin to hydrolyze pectin, thereby making tomato fruits more susceptible to microbial attack [17], and Wogu and Ofuase [18], reported that a broken tomato fruit can easily harbor pathogens that can spread and spoil all other tomatoes in the same environment. Table 3 presents the prevalence of bacterial flora isolated from tomato samples collected. Results obtained show that 85.71% of the samples analyzed are contaminated with *Escherichia coli*, 47.14% are contaminated with *Citrobacter spp*, 45.71% are contaminated with *Enterobacter cloacae*, 38.57% are contaminated with *Salmonella typhi* and 27.14% are contaminated with *Klebsiella pneumoniae*. These results therefore reveal that contaminated tomatoes could be rapid routes of transmission of foodborne pathogens to humans. Results of the evaluation of fungal contamination level in collected tomato (Table 4), also revealed the presence of fungi belonging to the genera of *Aspergillus*, *Fusarium* and *Mucor*. The most identified species are *Aspergillus ochraceus* (98.91%), *Fusarium oxysporum* (84.28%), *Aspergillus niger* (72.85%) and *Aspergillus versicolor* (35.71%). The presence of these fungi in collected tomato samples does not only represent a major risk of rapid deterioration of the marketable quality of contaminated tomato, but also a real risk for the health of the consumer, due to the fact that some of these isolated

fungi are susceptible to mycotoxins production. Bacterial and fungal contamination of tomatoes has also been reported in the literature. Indeed, Obeng et al. [19], revealed the bacterial contamination of tomato samples collected in Accra (Ghana), by bacteria such as *Proteus mirabilis*, *Enterobacter cloacae*, *Citrobacter koseri*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *Shigella spp.* Alemu et al. [1], also isolated from tomato samples collected in Ethiopia, some bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella spp* and *Salmonella spp.* In Nigeria, Pandukur et al. [20], reported the contamination of tomato samples collected from local markets, with pathogenic bacteria such as *Salmonella typhi*, *Erwina spp* and *Shigella spp.* Sajad et al. [21], reported fungal contamination of tomato samples collected in India by fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Mucor racemosus*, *Geotrichum candidum*, *Colletotrichum lycopersici* and *Rhizopus nigricans*. Shakya and Aryal [22], also reported fungal contamination of tomato samples collected in Nepal by *Alternaria alternata*, *Aspergillus solani*, *Aspergillus niger*, *Penicillium notatum*, *Phytophthora infestans* and *Rhizopus stolonifer*. According to Iwu and Okoh [11], foodborne pathogens are the significant causes of morbidity and mortality, especially in developing countries, and affecting approximately two million people each year. In these countries, the issue of food safety is increasingly becoming a public health problem, because of the microbial quality of foods [23]. Despite the nutritional importance of market garden products, food-borne illnesses linked to their consumption have increased considerably, resulting in a considerable burden on public health, and the multiple risks of epidemics associated [24]. “The raw or slightly processed mode of consumption of these foods explains why they are important vectors for the spread of pathogenic bacteria. In preharvest, irrigation water and manure-amended agricultural soil represent the two most important transmission pathways of enteric pathogens to fresh produce” [11]. However, taking into a count that enteric pathogens present in the soil contaminated with faeces and wastewater, are continuously introduced into the streams which are often used for crop irrigation, places irrigation water as the most important route of transmission of pathogenic microbes to vegetable products. According to Berger et al. [25], although parasites, fungi and viruses are implicated in foodborne illnesses, pathogenic bacteria are the main agents implicated in foodborne illnesses related to vegetable products. The pathogens mainly incriminated belong to the genus of *Escherichia*, *Klebsiella*, *Salmonella*, *Enterobacter*, *Citrobacter*, *Shigella* and *Listeria*, which are often associated with hemorrhagic colitis and dysentery (*E. coli* pathogens, *Shigella spp.*), sepsis (*Salmonella spp.*), miscarriage in pregnant women (*Listeria monocytogenes*), autoimmune complications and meningitis (*Enterobacter spp.*) [26]. The present study reveals that despite the potential

nutritional quality of tomato, abundantly reported in literature [27, 28], it is also subject to the contamination with microorganisms of public health concern as for other commodities marketed in the same conditions [29, 30]. At postharvest, this high bacteria contamination may also have been as a result of the low level of hygiene maintained during the sale of tomatoes. This includes the handlers and the open-air exposure of tomatoes in markets, which can serve as source of contamination by toxinogenic fungi and other food pathogens such as *Aspergillus ochraceus* isolated from analyzed tomato samples. These results pose a serious threat to food safety associated with tomatoes, due to the fact that sometimes, tomatoes are consumed without further processing. Therefore, special attention should be paid to the microbial quality of garden products in general and tomatoes in particular in order to preserve the health of consumers, especially those of the most vulnerable people such as children and in immuno-compromised people.

Conclusion

The contamination level of tomato and different bacterial and fungi species identified in this study can lead to food poisoning and food-borne illnesses and therefore raises concern on public health risks associated with the consumption of fresh tomatoes. Efforts should be made to advise farmers to avoid the use of contaminated wastewater for irrigation during cultivation, and discourage purchasing spoiled tomatoes, as well as avoid the consumption of raw or partially cooked tomatoes if possible.

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Table 1: Distribution of isolated bacteria in fresh and spoilt tomatoes samples analyzed

	Number of samples contaminated with each bacteria											
	<i>Escherichia coli</i>		<i>Citrobacter spp</i>		<i>Enterobacter cloacae</i>		<i>Salmonella typhi</i>		<i>Klebsiella pneumoniae</i>		<i>Proteus mirabilis</i>	
	G1 (n=5)	G2 (n=5)	G1 (n=5)	G2 (n=5)	G1 (n=5)	G2 (n=5)	G1 (n=5)	G2 (n=5)	G1 (n=5)	G2 (n=5)	G1 (n=5)	G2 (n=5)
Adjahonmin	4a	5a	1a	3a	2a	3a	2a	2a	0a	0a	0a	1a
Ayahohouè	3a	5a	2a	2a	1a	1b	2a	2a	0a	1b	2b	2a
Djotto	4a	4a	0b	0b	1a	2b	0b	1a	0a	2b	1b	3b
Hondjin	2b	5a	4c	4c	1a	3a	1a	1a	2b	2b	0a	0c
Klouékanmey	4a	5a	3c	5c	2a	4a	3b	3b	2b	3c	0a	2a
Lanta	4a	5a	2a	3c	3b	5a	2a	4b	0a	2b	2b	3b
Tchikpé	5a	5a	2a	2a	1a	3a	2a	2a	0a	1b	1b	2a

Values are mean. Means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

- G1: Fresh tomato

-G2: Spoilt tomato

Table 2: Distribution of the contamination rate (%) of the different tomato samples analyzed according to isolated bacteria

	<i>Escherichia coli</i>	<i>Citrobacter spp</i>	<i>Enterobacter cloacae</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>
Fresh tomato samples	74.28a	35.0a	31.42a	34.28a	11.42a	17.14a
Spoilt tomato samples	97.14b	54.28b	60.0b	42.85a	31.42b	37.14b

Values are mean. Means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 3. Bacteria isolated from tomatoes samples

Bacteria	NCI	Occurrence (%)
<i>Escherichia coli</i>	60	85.71 a
<i>Citrobacter spp</i>	33	47.14b
<i>Enterobacter cloacae</i>	32	45.71b
<i>Salmonella typhi</i>	27	38.57b
<i>Klebsiella pneumoniae</i>	19	27.14b
<i>Proteus mirabilis</i>	15	21.42b

Values are mean. Means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

NCI. Number of cases of isolation out of 70 samples

Table 4. Fungi isolated from tomatoes samples

Fungi	NCI	Occurrence (%)
<i>Aspergillus ochraceus</i>	69	98.91 a
<i>Fusarium oxysporum</i>	59	84.28a
<i>Aspergillus niger</i>	51	72.85a
<i>Aspergillus versicolor</i>	25	35.71b
<i>Mucor spp.</i>	4	5.71c

Values are mean. Means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

NCI. Number of cases of isolation out of 70 samples