

## Incidence of Salmonella and E. coli in Mango exporting company in the State of Nayarit, Mexico

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### Abstract

The following investigation analyzed conditions of hygiene on that operate in mango fruit packer companies in Nayarit, for it we were in contact with someone in the Mpio de Santiago Ixcuintla, main exporter of mango, according to production statistics in 2010. Samples of 11 places took: 1) the conveyor belts, 2) hands of workers who place mangos in plastic boxes, 3) packers hands, 4) walls of boxes of plastic, 5) walls of cartons where mango is packaged, 6) wash water, 7) hidrotermic treatment of water and 8) hidrofrezzed water, 9) field mango, 10) handle previous to the treatment hidrotérmico and 11) packaged mango. The methodology used to detect Salmonella was by means of rapid Tecra UNIQUE™ test by means of which it allows the Salmonella detection in 16 -18 hours, sufficient time to stop handle shipments before the arrival to the commercial destination. For E.coli detection and coliformes it was realized by means of Badges of inventories of Petrifilm

We found presence of Salmonella in 10/11 sampling places, being the hands of the workpeople the point of major incidence, E. coli was detected in major percentage in classifiers hands, at the beginning of the flow aswell as the packers' hands at the end of the handling, with values of more than 10<sup>5</sup> Log. The target of the investigation was to detect the main sources and contamination indexes of pathogenics like Salmonella and E. Coli on the epicarpio mango, in hands of workers and surfaces inside an exporting company of handle.

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**Introduction**

The United States is the main importer of handle (Indian *Mangifera L.*) in the world, and Mexico is its main provider. Nevertheless, the participation of Mexico in the imports of handle in the American market has diminished, at the time that Peru, Brazil and Ecuador have increased its participation. (Hernández-Soto et al., 2009).

Nevertheless, in the last years the segment of major growth inside the food industry is that of fruits and vegetable fresh cut (FVFC) what has increased the investigation of this title substantially. As well as between the tropical fruits major potential on the market of (FVFC) there meets the handle due to its high world production as well as for the preference of the consumer to the high nutritional value (González-Aguilar et al., 2008).

Also, the handle is one of the fruits of major consumption in Mexico., being provided with an approximate surface of 146 000 There is and an approximate production of 1.34 million tons of which only there is exported of 12 15 % of the whole of the production (Wong et al., 1997).

The varieties of major exportation are "Ataulfo", "Tommy Atkins", "Haden", "Kent" and "Keitt" (SAGARPA, 2010).

The quality is a key factor in the commercialization of the handle since the consumers demand every time higher standards, so much in the parameters físicoquímicos related to the ripeness, as well as in its external appearance (Lady's man, 1992).

The mango fruit, as all other fruits and vegetables, continues its biochemical and physiological processes after still being harvested, on having induced the fruit's maturity and later the aging. Some of these processes include changes in the color, increase in the sugar content, pH and the relation sugar / acid, as well as the reduction in the steadfastness, acidity and weight. Several of these characteristics are used like indicators of the state of ripeness and of the quality of poscosecha of a fruit (Hulme, 1971; Seymour et al., 1990; Baez, et al., 1998).

Many studies were based on the indicated variables put together with the temperatures of storage of the fruits only on the effect that they induce in the quality of the fruit of handle, and its life of shelf (Thomson, 1971; Thomas, 1975; Seymour et al., 1990); without considering the harmlessness of the product. By what it tries to be contributed which are the places of major danger where the mango could be contaminated during its handling in the packing, already being contaminated by the physical ambience or for contact with food manipulators. In addition to that, this fruit presents a hidrotermic treatment to an established temperature of 46.1°C during 75 to 90 minutes of exhibition, depending on the variety of the mango. In case of less fruits of 500g he is given a time of 75 minutes (as in the handle Ataulfo) and in fruits bigger than 500 g (I marry handle Kent and Keitt) it is exhibited for 90 minutes.

These exhibition times to major temperatures of the temperature average of the fruit, (25 °C), alter the physiological and biochemical properties of the handle, bearing to the increase of weight, of temperature, change of color, of steadfastness, and of processes enzimáticos (Osuna, et to the, 2005; Osuna et to the, 2007; Chaidez, et to the, 2002; Ibarra, 2006).

All these factors can favor the contamination microbiana, to its adhesion and its internalization (Ibarra, 2006).

The target of the present study was to detect the main sources and contamination indexes of pathogenic like Salmonella and E. Coli on the handle epicarpio, in hands of workpeople and surfaces inside an exporting company of mango.

### **Materials and methods**

Tracking of the sources of contamination with Salmonella and E.coli that operate in the facilities of a baler of mango in the Edo. of Nayarit, Mexico.

It made a trace of Salmonella and E. coli that was carried out during a harvest season of the handle in the state of Nayarit (May-August), in a company baler located in the MPIO. Of Santiago Ixcuintla.

Samples were taken of 11 points within the company baler , among which are: 1) the conveyor belts, 2) hands of workers that placed the handles in the plastic crates, 3) hands of packers, 4) walls of the plastic boxes, 5) walls of the cardboard boxes where is packed the mango, 6) the washing water,

7) Water of the hydrothermal treatment and 8) the water of the hidrofriado, 9) field handle, 10) mango before of the hydrothermal treatment and 11) mango baling. Samples 1 to 5 and 9 to 11 were taken with a sponge sterile (brand Bio Pro Sampling System) which were previously moistened with water peptonada sterile 0.1% Vigorously rubbing surfaces of interest.

The samples of water (6 to 8) were taken by a Moore swab (Sepulveda, 2002) which consisted in a portion of gauze of 30 X 30cm, rolled up on itself to form a cylindrical strip approximately 10X4, is sewed at both ends with a thick thread, and on a shore left a piece of wire, with which was later joined by a piece of raffia for secure in a shore tank of the hydrothermal treatment. The swabs were sterilized previously wrapped in paper dextrasa, and once placed the swabs were left in the respective vats of washing, of hydrothermal and hidrofriado by approximately 5 hours and then withdrew and were placed in a sterile pouch, adding pre-enrichment broth, to be transported to the laboratory, like the other samples, within a cooler with refrigerants.

The sampling of the hands of the workers was to rub the hands of 5 workers with the sponge moistened in the broth of preenriquecimiento and in this way, with each sponge is took a pool of 5 samples which counted as a single.

For the sampling of the mango, of each sample was prepared a pool by sampling the surface of 10 handles in the different stages:

Handles newly received from the field, another of mangos before hydrothermal treatment and hidrofriado and finally of mangoes packaged.

The 11 points of sampling were studied in 25 different occasions, which meant that it took a total of 275 samples that were obtained during the whole season of mango production (May-August), becoming daily sampling until completing the sample size. To distribute the sampling in 25 occasions, broadened the possibility of finding Salmonella in those sites, as the conditions of work could vary from one day to another.

Once taken all samples, were kept in a cooler with coolant to be retained until processing. The same day of sampling were transferred to the laboratory and began with the detection of Salmonella and E. coli. The detection of Salmonella was carried out by the test of Tecra UNIQUE TM and the detection of E. coli and coliforms was carried out using rapid tests of Petrifilm (plates of E. coli count and coliforms) 3M.

The technique for detecting Salmonella for this stage of the work was carried out by the test Tecra UNIQUE TM, this method is equivalent to the method of reference of US FDA BAM.

### **Detection of Salmonella with the Tecra UNIQUE TM Test**

Each 25 ml of the sample was added 225 ml water peptonada damped (Tecra, MBPMED500).

As a means of pre-enrichment and were incubated at 35°C for 18 to 24 h. After that time of incubation, proceeded to remove the cap from the pipes 1 to 3 of the test of Tecra UNIQUE TM and transferred 1 ml of enrichment broth to tube 1 test, putting the paddle on the inside of the tube 1 with the numbered part toward the pipe 2. Mixed 2 times and incubated for 20 to 40 minutes at 35°C. During this time are captured some Salmonella present using the purified antibodies highly specific present on the surface of the palette. Subsequently, was transferred the palette to the tube 2 by washing 4 times and was passed to the pipe 3 and incubated again during 4 h, at a temperature of 35°C where it happened replication of Salmonella, which multiplied on the surface of the palette and produced a sufficient number to achieve the detection. Removed the pipe cover 4 to 6 and the palette is change to the tube 4 and was incubated for 30 minutes at 35°C. In this step is presented a conjugate, since the tube 4 contained enzymes linked to specific antibodies for Salmonella (conjugate). The Palette is transferred to the tube 5 where they washed 5 times; then change to the tube 6 and incubated at a temperature of 20-25°C (ambient temperature) for 10 minutes. The tube 6 contained a substrate for the enzyme. If Salmonella was present, was developing a violet color on the bottom of the palette. Finally, they took the results by placing the lever in the front of the support of the test and the color is compared with the color chart included in the test.

In the case of the positive samples, took a roasted pipe 3, which contained broth M and was planted in agar plates agar Hektoen sulphite bismuth, and XLD agar and incubated at 35°C during 24 to 48 h. The colonies that were suspected in these media, they practiced biochemical tests (agar triple sugar iron (IST), Hagar lysine iron (LIA) and urea incubating at 35°C during 18 to 24 h. The tests that were positive for Salmonella were confirmed with evidence of agglutination with polyvalent antiserum.

It should be noted that the technique for detecting Salmonella used in this paragraph by the quick test of Salmonella Unique TM allows only make a qualitative assessment of the bacteria detected

### **Identification of E. coli**

Of the samples taken for Salmonella, took 1 ml and was placed in Petrifilm plates for E. coli and Coliforms, previously labeled for each one of the samples, planting 3 plates; the first was a direct planting, the second a dilution 10-1 and the third a dilution 10-2 incubating at 35° for 24 h, at the end they were practiced counts of blue colonies with gas and coliforms.

### **Results and Discussion**

**Detection of sources of contamination with Salmonella and E.coli that operate in the facilities of a baler of mango in the Edo. of Nayarit**

**Detection of Salmonella in packaging of mango**

The presentation of the results of the frequency of Salmonella has the order of workflow within the company baler, since mangos arrive at the packaging until this is packaged for your shipment.

The percentages of salmonella found in the 11 sites sampled within a company baler mango (Graph 1) were the following, being the hands of workers who are manipulating the handle before treatment of washing presented the highest percentage of contamination (32%), followed by the gangs that transported to handle after being washed and treated hidrotermicamente, (20%). The rinse water from the company owns 16% of frequency of contamination with Salmonella, like mangoes which come from field (16%). The handle in the stage prior to the hydrothermal treatment like the cardboard boxes where is packed the handle have a (12%) of contamination percentage of the total sampled, the hands of the packers that are at the end of the line of hydrothermal treatment and hidroenfriado sampled along with the handle baling presented 4% of contamination with Salmonella.

### **Detection of E. coli in sites sampled in mango packaging**

The Graph 2 shows the data obtained in the detection of E. coli in the 11 sites sampled presenting great variation of the populations in each one of them, fluctuating between 101 and 105 CFU/ml. It should be noted that the order presented in graphs 3, 4 and 5 is the same that exists in the flow of the processing of the handle in companies balers.

The highest counts of *E. coli* are presented in the hands of workers who handle the handle before washing and from the hands of the packers, that is the last step in the process of the packaging of the fruit, with populations of 105 CFU/ml. The conveyor belts represent the second site of greater presence with *E. coli* with counts of 103 CFU/ml. The rest of the sampled sites are in a range of populations of this bacteria of 101 and 102 CFU/ml.

### **Detection of coliforms in sites sampled in mango packaging**

The Graph No. 3 reveals the high concentrations of coliforms in the majority of the sampling points, with populations of 106 CFU/ml and the water that is used in the hydrothermal treatment is the one that contains a lower level of coliforms (103CFU/ml).

The hands of the workers who represented the first place of contamination with *Salmonella*, it may be because they are the first to have contact with the fruit that comes from field, there is a high probability that the handle that they play is contaminated with this pathogenic bacteria in addition to contaminate the objects that have contact as the plastic crates, cartons, or other.

There is the possibility that workers are asymptomatic carriers of *Salmonella*, or that only due to poor hygiene practices and do not have a correct washing hands before start the day and after going to the bathroom or when removed from their activities, causing the return have dirty hands and carriers of salmonella.

This situation is in accordance with the studies of (Cruickshank, 1990), who asserts that a proper handwashing decreases the risk of foodborne diseases by asymptomatic handlers.

In some studies reveal that the infected food handlers arrive to cause in the United Kingdom an average of 7 per cent of outbreaks of foodborne diseases (Bryan, 1978) and (Brayan, 1998).

In addition, the Center for Disease Control of the United States, (FDA, 2000) estimated that 20% of events of foodborne diseases caused by bacterial agents are a result of transmission from infected workers.

Certain authors as (Bean & Griffin, 1990; Bean et al, 1996; Olsen et al, 2000) indicate that there is data in the United States that from 1973 to 1997, the poor personal hygiene of workers of foodstuffs has been the second factor frequently cited that contributes in outbreaks of ETAs.

The Conveyor Belts represent the second place of pollution in the company baler, perhaps due to contact with the workers, who can contaminate it, and also by the constant friction that have with handles that are happening throughout the working day, which can present contamination with *Salmonella*.

Because *E. coli* has been widely used as an indicator of faecal contamination (Geildrech, 1966), this microorganism was monitored within the company baler mango, with the object of check in an indirect way the presence of *Salmonella*.

And when considering to *E. coli* as an indicator organism, when analyzing the results is that the hands of the workers, both of which receive the handle from the field for a preselection and washing and from the hands of the packers are the main sources of fecal contamination that may contaminate the existing equipment and the boxes that are used to position the handles washed and the boxes where they are packaged.

The large numbers of *E. coli* in the hands of the packers can arise due to the workers of the company in its most women, avoid washing hands during the working day, even when removed from their activities because they concern that this causes them reumas to wash your hands. This can lead to a serious problem of safety for the fruit. In addition, workers do not have a training to make a correct washing hands, and there is no adequate monitoring so that there is always soap and role of drying in the laver. Michaels et al, (2004) notes that factors such as a poor personal hygiene, bad hand washing and drying inappropriate coupled with inadequate facilities have been documented that contribute to cause foodborne diseases.

With regard to the water used in the company although the numbers of *E. coli* are low in concentrations of 101 and 102 CFU/ml, represent a potential risk of contamination in the epicarp of mango, since the fruit is kept in contact with the water, may become contaminated. The rinsing water, but these are only 15 to 20 sec of exposure, are sufficient for some bacteria can remain on the surface to pass through the rolls of washing, or even could infiltrate (Ibarra et al, 2004).

The waters of hydrothermal and hidroenfriado although present lower concentrations of *E. coli* of 101 CFU/ml, represents a greater risk due to the exposure time of the fruits of mango in the tanks of the hydrothermal water are 46.1°C during 75 to 90 minutes depending on the variety of fruit and in tanks of hidroenfriado at ambient temperature are 20 minutes, time enough to provoke an infiltration of water to the inside of the handle, in the area of the stalk, as it was found in the study of Ibarra et al (2006) in which there was infiltration of water during the hydrothermal and when applied both treatments, presented a higher percentage of infiltration due to the temperature differential Between the hidrotermico e hidroenfriado, 21°C, and mangoes hot (46°C) to be immersed in water at ambient temperature (25°C), there is a contraction of gases from the interior of the fruit, causing a suction to the interior. And here is the problem that if the water is contaminated with bacteria, these not only can contaminate the surface, but infiltration to the pulp of the mango (Ibarra, 2006), (Ibarra et al, 2004)

### **Conclusions**

It is necessary to know the events that occur during the cultivation of mango, harvest and transport of mango, before this arrive to packaging, which favor the contamination of fruit with pathogenic bacteria, as in the present study there was evidence of contamination of Salmonella, *E. coli* and coliforms in the entire surface of the handle when this comes from the field to the company baler.

The current systems for washing and disinfection of fruit and vegetables in the exporting companies of fruit and vegetable products, are insufficient to ensure the safety of the fruit. These technologies were designed to remove the debris from the products, not the microorganisms. And even with new knowledge of disinfectants as well as techniques, it is preferable to avoid microbial contamination through Good Agricultural Practices and Good Management Practices rather than relying on decontamination technologies.

It is known that depending on the time interval between the contamination of the surface of the fruit and vegetables with pathogens, varies the efficiency of the decontamination treatments applied and the greater the interval time, there is a greater probability of smaller reductions logarithmic and possibility of adherence and bacterial internalization within the products. In addition, the cleaning equipment used in the exporting companies do not allow a sufficient contact between the adhered bacteria on the surface of the fruit and vegetable products and agents of washing and sanitizantes with the wash brushes. In addition, brushing during washing can cause injury to the entire surface of the handle, imperceptible to the view, which can promote the internalization of pathogens.

In addition to the above, sanitizantes as hypochlorite, commonly used in the water for processing fruit and vegetables to decrease the bacteriological population, are inefficient, as this item is quite unstable.

Especially in the presence of organic matter and when this is applied to high temperatures such as is the case of the hydrothermal treatment (46.1°C) evaporates with ease and in less than one hour after being applied is reduced fully its concentration. And although there are some alternative sanitizantes to eliminate pathogenic bacteria on the surface of fruits and vegetables, have not yet been adopted by the majority of the companies from the orchard.

To achieve an assurance of the safety of fresh fruits and vegetables is necessary a management system that integrates all aspects of production, processing and distribution, and more training to workers who work both in the field and in the food plants in terms of hygiene and safety. The facilities should be suitable to avoid the entry of birds, rodents, domestic animals and wildlife as iguanas, among others, which represent a potential source of contamination with Salmonella.

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