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CAUSE DETECTION AND DECREASE OF QUARANTINE HOT WATER TREATMENT INJURY IN 'ATAULFO', 'TOMMY ATKINS' AND 'KENT' FRUIT GROWN IN NAYARIT

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CONTENT

Executive summary ……………………………………………………………………2
Background ……………………………………………………………………………4
Objectives …..…………………………………………………………………………6
Methodology ……..……………………………………….……………………………6
Detailed description of methodology ……………….…………………..…………..7
Results and Discussion ……………………………………………………………… 9
Full cycle Packinghouse 1. Ataulfo ………………….……….……………….……. 9
Physical and chemical quality of processing water…..………………..…………… 9
Microbiological quality of processing water ……………..…..………………………10
Microbiological quality on contact surfaces …………..…………………………… 12
Fruit quality ………………………………………..……………………………… 13
Scanning Packinghouse 2. Tommy Atkins……….…………………………........16
Physical and chemical quality of processing water…..………………..……………16
Microbiological quality of processing water ……………..…..………………………18
Microbiological quality on contact surfaces …………..……………………………20
Scanning Packinghouse 3. Kent ………..……….……………………………........22
Physical and chemical quality of processing water…..………………..……………22
Microbiological quality of processing water ……………..…..…………………… 24
Microbiological quality on contact surfaces …………..……………………………26
Preventing contamination in processing water ………………………………………28
Effectiveness of chloride as a disinfectant ……………………………………...28
Determination of chloride injury ..……………….…...…….……………………… 30
Effectiveness of chlorine concentrations on washing water with prolonged use….33
Diagnosis of 'Black Cut' and 'Spongy Tissue' in Ataulfo variety …………………......37
Conclusions …….……………………………………………………………………… 44
References ……………………………………………………………..….…………45

EXECUTIVE SUMMARY

Mexico is one of the main mango exporters to the USA sending 65.6% of the total volume representing around 57.5 million boxes by year. With a few exceptions, the USA requires quarantine hot water treatment (QHWT) to control fruit fly larvae. This QHWT consists of treating the fruit with hot water at 115°F for 65 to 110 min, depending on the fruit size. If this treatment is applied according to the protocol, it does not cause any damage to mango fruit. However, during the packing process the fruit may be susceptible to contaminate, especially in the QHWT, where there is no application of disinfectants because the high temperatures deactivate them. It is speculated that growth of some pathogens and their damage to mango fruit may be due to deficiencies in the water used for QHWT and hydrocooling. In fact, fresh mango consumption has been pointed as a cause of outbreaks of foodborne diseases, even with fruit coming from certificated packinghouses. It demonstrates that something is failing during the packing process and some handling practices should be improved to decrease the risk of microbiological contamination. Thus, the objectives of this study were: a) to determine the chemical and microbiological quality of the water used for QHWT or hydrocooling at the beginning, middle, or at the end of a treatment cycle b) to define the effect of water quality on initial quality, shelf life, and quality at consumption time of 'Ataulfo', 'Tommy Atkins' and 'Kent' fruit submitted to QHWT and/or hydro-cooling, and c) to establish if the prolonged use of water for QHWT or hydrocooling is a vehicle for the survival of pathogenically microorganisms that cause foodborne diseases or postharvest diseases like anthracnose (*Colletotrichum gloeosporoides*) and soft rot (*Lasiodiplodia theobromae*). Other objectives were: to determine chlorine effectiveness as a disinfectant and its effect at high concentrations on fruit damage and to determine only in 'Ataulfo' if ripening degree and duration of refrigerated shipping were correlated to 'Corte Negro' and 'Spongy tissue'.

To fulfill the first three objectives two types of assays were done: a) Full cycle in the packinghouse 1 with 'Ataulfo', and b) Scanning in packinghouses 2 and 3 with 'Tommy Atkins' and 'Kent', respectively. For a full cycle (considering from the beginning to end of a hydrothermal or hydrocooling treatment) sampling was taken from both tanks at the beginning (first basket), in the middle (seven baskets), and at the end (14 baskets). In all the cases, water samples were taken from the source, QHWT tank and hydrocooling tank for physical, chemical and microbiological analysis. In addition, contact surfaces of field boxes, packinghouse boxes, belts, benches, and packed fruit were included for analyzing *total coliform* and *total aerobic bacteria*. Finally, fruit quality variables were measured, considering dry matter, weight loss, skin color, firmness, pulp color, total soluble solids, tritatable acidity, and ratio °Bx/Acidity, as well as development of phytopathogens: *Colletotrichum gloeosporoides* and *Lasiodiplodia theobromae*. For scanning, a similar methodology was used, but in tanks with a different level of use. Since some contamination was found in boxes and fruit becoming from the field, it was decided to set a control in the washing water. Three experiments with a commercial disinfectant were designed: a) Effectiveness of chlorine as a

disinfectant; b) Determination of injury by chlorine, and c) Effectiveness of chlorine in water with prolonged use. For 'Black cut' and 'Spongy tissue' activities, three surveys were done at the beginning, middle and at the end of 2016 harvest season for 'Ataulfo'. In each survey, 150 partially ripe fruit and 150 ripe fruit were selected. Both groups were submitted to similar QHWT and hydrocooling and then stored at 53.6 ± 1.5°F; 90 ± 5% HR for 1, 2, or 3 weeks + market simulation (71.6 ± 3°F; 75 ± 10% HR) until consumption stage. Quality variables and percentage of fruit with 'Black cut' and 'Spongy tissue' were recorded.

Results showed that for water quality, turbidity was the most affected variable, which increased according the prolonged use favoring microbial growth. In QHWT tanks, it is suggested to change the water no longer than 14 baskets by cycle. Besides that, the water with prolonged use in washing or hydrothermal tanks presented *total coliforms* and *total aerobic bacteria* indicating microbial contamination risk. However, if the water in the hydrocooling tank is maintained at 20-50 ppm chlorine, the pathogen microorganisms are controlled. In addition, it was found that the most critical point for fruit contamination in contact surfaces was field boxes, which should be washed and disinfected before returning to the field. On the other hand, no organoleptic differences were found among fruit coming from hydrothermal or hydrocooling tanks nor packed boxes. Concerning to the use of chlorine as a disinfectant, it was effective at 20 ppm to eliminate *total coliforms* and *total aerobic bacteria*; the initial chlorine concentration should be 200 ppm, since it brings down until 10 ppm at the end of a washing cycle with 600 boxes of mango fruit. The chlorine in water with prolonged use should be used no more than three cycles but the recommendation is to change the water of washing tanks every cycle of 600 boxes. Finally, it was detected that the presence of 'Black cut' and 'Spongy tissue' was practically inappreciable in the three surveys done during the 2016 harvest season. Neither the ripening degree nor the duration of refrigerated storage affected both physiological disorders. On the other hand, results of this experiment let us to confirm the effect of long-term conventional refrigeration on main quality variables. At the end of refrigeration for one, two, or three weeks, the weight loss increased, the skin color and pulp firmness decreased, and the development of total soluble solids and pulp color was accelerated.

BACKGROUND

Mexico is one of the main mango exporters to the USA sending 67% of the total volume representing around 60 million boxes by year (USDA-FAS, 2012). The main varieties are Tommy Atkins, Ataulfo, Kent y Keitt with 35, 30, 15, and 10% respectively of the total exported volume (EMEX, A.C., 2014). The USA requires quarantine hot water treatment (QHWT) to control fruit fly larvae. This QHWT consists of treating the fruit with hot water at 115 °F for 65 to 110 min, depending on the fruit size (USDA, 2010). Recently in a study financed by the National Mango Board (NMB) Osuna et al. (2015) demonstrated that if QHWT is applied according to the USDA protocol, no injury should be observed in 'Tommy Atkins' fruit. However, in another assay financed by the NMB, Osuna-García (2014) found that 'Ataulfo' sampled at the beginning, middle, or by the end of the harvesting season fruit submitted to QHWT and hydrocooling showed external damage at the end of seven days of simulated refrigerated shipment (12 ± 1°C; 85 ± 10% HR), or at consumption time. In addition, in another test where the aqueous 1-MCP effect (400, 800 y 1200 µg L^{-1}) was evaluated in 'Keitt' mango fruit, it was found that fruit treated with QHWT and hydrocooling showed stem end rot (*Lasiodiplodia theobromae*); while those without QHWT exhibited anthracnose.

There are techniques to reduce the microbial growth of fruit during the packing process. Washing the fruit in a chlorine solution (100-200 ppm) or another effective disinfectant may reduce microbial contamination. However, during the QHWT no disinfectants are used since the high temperature (115 °F) deactivated them. The QHWT inhibits the growth of most microbial pathogens in fruit while killing the fly fruit larvae, which is the main objective (Spalding et al., 1998). Nevertheless, this hot water treatment may compromise the quality and safety of mango fruit because when the hot fruit is cooled down with cold water, it shrinks creating an inner hydrostatic strength, which sucks out microbial pathogens present in the water causing contamination of the fruit that may origin human health risks (Silva Beltrán *et al*., 2004).

Fresh mango consumption has not escaped the attribution to cause outbreaks of foodborne diseases even from packinghouses certified under systems of reducing contamination risks. In 2001, an outbreak of *Salmonella* in several US states was associated with the consumption of fresh mango from Peru, because of the inadequate chlorination of water for washing the fruit. In addition, in 2012, *Salmonella* serotype Worthington and Braenderup was linked with an outbreak of 127 infections in Canada and USA (www.outbreakdatabase.com).

This shows that at some point of the production process and packing of exporting mango, something is failing. Therefore, handling practices to reduce the risk of contamination should continue improving. In this regard, Osuna *et al*. (2010) state microbiological quick methods are an excellent alternative to establish controls for frequent monitoring of hygiene during the packing of exporting mango.

There is speculation that the presence of physical, chemical, and microbiological contaminants, as well as the growth of some pathogenically microorganisms and the consequent injury to mango fruit may be due to deficiencies in the chemical and microbiological quality of the water used for the QHWT and hydrocooling. In fact, the problem is worst especially for the last treated baskets since there is not a uniform criterion to determine the ideal time to change the water in a same basket for QHWT or hydrocooling. Regarding that, some mango packers treat 9 to 12 baskets with 180 boxes before changing the water, while others proceed to change the water treating up 37 baskets. It is logic to expect that the more baskets treated, the higher the microbiological contamination risks. In addition, because of soil accumulation and organic residues, the incidence of postharvest diseases like anthracnose (*Colletotrichum gloeosporoides*) and soft rot (*Lasiodiplodia theobromae*) increases. However, those packers treating a few numbers of baskets reduce the contamination risks, but they could be increasing costs and wasting water, a scarcer resource every day. Until now, there are no studies that determine the optimum balance from the biological and economical point of view to minimize the contamination risks, as well as, to optimize the use of resources.

OBJECTIVES

- To determine the chemical and microbiological quality of the water used for QHWT or hydrocooling at the beginning, middle, or at the end of a treatment cycle.
- To define the effect of water quality on initial quality, shelf life, and quality at consumption time of 'Ataulfo', 'Tommy Atkins' and 'Kent' fruit submitted to QHWT and/or hydrocooling.
- To establish if the prolonged use of water for QHWT or hydrocooling is a vehicle for the survival of pathogenically microorganisms that cause foodborne diseases or postharvest diseases like anthracnose (*Colletotrichum gloeosporoides*) and soft rot (*Lasiodiplodia theobromae*).

METHODOLOGY

a. **VARIETIES:** Ataulfo, Tommy Atkins and Kent.

b. **PACKINGHOUSES:**

   Packinghouse 1: Ataulfo

   Packinghouse 2: Tommy Atkins

   Packinghouse 3: Kent

c. **FRUIT SAMPLING STEPS:** Collect a 50-fruit sample (completely randomized) in the following steps: 1. After QHWT at the beginning, middle, or at the end of a treatment cycle. 2. After hydrocooling at the beginning, middle, or at the end of a treatment cycle.

d. **WATER SAMPLING STEPS:** Collect water samples at the same time of fruit sampling (at the beginning, middle, or at the end of a treatment cycle) to determine physical, chemical, and microbiological water quality.

e. **FUIT STORAGE:** Simulation of refrigerated shipment (Seven days at 12 ± 1°C; 90 ± 5% RH) + Market simulation (22 ± 2°C; 75 ± 10% RH) until consumption stage (full color fruits with pulp firmness of 1 to 3 Lbf).

f. **SAMPLING:** Initial, at the end of refrigerated period and then at consumption stage.

g. VARIABLES MEASURED

1. **FRUIT:** Dry matter, weight loss, external appearance, skin color, firmness, pulp color, total soluble solids (ºBx), tritatable acidity, and ratio ºBx/acidity, as well as presence/absence of Enterobacteria (*Salmonella*, *total Coliforms* and *E. coli*), and development of phytopathogens (*Colletotrichum gloeosporoides, Lasiodiplodia theobromae*).
2. **WATER:**
   - Chemical quality: Turbidity (< 5UNT), chlorides (< 250 mg/l) total hardness as $CaCO_3$ (< 500 mg/L), Nitrates (< 10 mg/L), Sulfates as SO_4 (< 400 mg/L), total dissolved solids (< 1000 mg/l) and pH (6.5-8.5) [NOM-127-SSA].
   - Microbiological quality: Presence / absence of *total coliforms.*
   - Development of phytopathogens: *Colletotrichum gloeosporoides* and *Lasiodiplodia theobromae.*

A Factorial design was used with 20 replications for weight loss, three for water samples and eight for all the other fruit variables.

Detailed description of methodology

For packinghouse 1, three samplings were done at the beginning, middle, or at the end of 2016 mango season. In each of them a full cycle for a QHWT and a hydrocooling tank was done at the beginning (first basket), in the middle (seven baskets), and at the end (14 baskets). In all the cases, water samples were taken from the source, QHWT tank and hydrocooling tank for physical, chemical and microbiological analysis. In addition, contact surfaces of field boxes, packinghouse boxes, belts, benches, and packed fruit were included for analyzing *total coliform* and *total aerobic* bacteria. Finally, fruit quality variables were measured, considering dry matter, weight loss, skin color, firmness, pulp color, total soluble solids, tritatable acidity, and ratio °Bx/Acidity, as well as development of phytopathogens: *Colletotrichum gloeosporoides* and *Lasiodiplodia theobromae*.

Because some contamination risks were detected, three experiments to control water contamination were set: 1. Effectiveness of chloride as a disinfectant (0, 10, 20, 30, 40, and 50 ppm); 2. Determination of chloride injury (0, 50, 100, 150, and 200 ppm), and 3. Effectiveness of chloride in water of prolonged use.

By request of Dr. Leonardo Ortega, Research Director of the NMB, an additional activity was included only in the Ataulfo variety. This activity tried to determine if ripening stage at harvest and length of refrigerated storage have effect on the presence of the anomaly called 'Black cut'. To do that, in each of the three sampling at the beginning, middle, or at the end of 2016 mango season from a same lot, 150 partially ripe fruit (flat cheeks, pulp color 1 to 2, and total soluble solid content < 6.0 °Bx) and 150 ripe fruit (full cheeks, pulp color 2 to 3, and total soluble solid content > 6.0 °Bx) were collected. Both groups were submitted to similar conditions of hydrothermal and hydrocooling treatment and then kept under refrigerated storage (12 ± 1°C; 90 ± 5% RH) for 1, 2, or 3 weeks + Market simulation (22 ± 2°C; 75 ± 10% RH) until consumption stage.

The variables to measure were: dry matter, weight loss, external appearance, skin color, firmness, pulp color, total soluble solids (ºBx), tritatable acidity, ratio ºBx/acidity, and percentage of fruit with 'Black cut'. Sampling was done at the initial, at the end of refrigerated period and then at consumption stage. A completely randomized design with 20 replications for weight loss and 10 for all the other fruit variables was used.

For packinghouse 2 and 3, the same number of samplings as packinghouse 1 was done; the only difference was that in these packinghouses the sampling was done in one day, as a scanning including from fruit reception until packed fruit in Tommy Atkins and Kent varieties, respectively. The same variables for fruit and water were measured.

RESULTS AND DISCUSSION

1. Full cycle Packinghouse 1. Ataulfo.

a. Physical and chemical quality of processing water.

Results showed the most affected variable was turbidity (Table 1), which increased its value according to the number of baskets and exceeded the levels allowed by the Standard (NOM-127-SSA, 1994) only in the case of hydrothermal water (QHWT). Turbidity is an indicator of contamination in water because it implies the existence of substances or microorganisms that can cause some damage to health. The literature mentions that turbidity causes the bacterial content to increase, since UV rays do not manifest their action. In the Figure 1, it can be seen how the turbidity of hydrothermal water increases as the number of baskets increases, so the suggestion is to change the hydrothermal treatment water to no more than 14 baskets per cycle.

Table 1. Physical-chemical composition of the processing water in exporting mango packinghouses during a complete cycle.

	Initial (1)		Middle (7)		End of cycle (14)	
Treatment	pH	Turbidity (UNT)	pH	Turbidity (UNT)	pH	Turbidity (UNT)
Source	7.7	0.3	7.7	0.7	7.7	0.3
Washing	7.6	0.3	7.6	1.0	7.6	0.3
QHWT	7.3	7.0	7.2	11.3	7.3	19.0
Hydrocooling	7.2	1.3	6.7	1.0	6.8	1.7
NOM-127-SSA	6.5 - 8.5	< 5.0	6.5 - 8.5	< 5.0	6.5 - 8.5	< 5.0

Figure 1. Illustration of turbidity of processing water for exporting mango packinghouses.

b. Microbiological quality of the processing water.

Table 2 shows the results of the microbiological analysis in source, washing, hydrothermal and hydrocooling water. It was detected the presence of *total coliforms* in washing and hydrothermal water, even from the beginning of a complete cycle. The foregoing indicates a pollution hazard that must be addressed to reduce the risk. On the other hand, when sampling hydrocooling water with a chlorine concentration of 20 to 50 ppm, it was observed that concentration was effective to control the presence of *total coliforms*. Therefore, the recommendation for all packers is to maintain a chlorination level of 20 to 50 ppm in hydrocooling water tanks.

Table 2. Presence/Absence of *total coliforms* in process water for exporting mango packinghouses.

	Initial (1)	Middle (7)	End of cycle (14)
Treatment	*Total coliforms*	*Total coliforms*	*Total coliforms*
Source	-	-	-
Washing	+	+	+
QHWT	+	+	+
Hydrocooling	-	-	-
NOM-127-SSA	-	-	-

(+) Presence (-) Absence

Figure 2 shows the result of the sampling for Presence/Absence of *total coliforms*. At the end of 48 h of incubation, the washing and hydrothermal water samples were positive in the presence of *total coliforms*, indicated by the color turning from purple red to yellow. In contrast, samples of hydrocooling water were negative for the presence of *total coliforms* since they did not change color and as indicated above, due to the concentration of chlorine from 20 to 50 ppm, which completely inhibited the presence of *total coliforms*.

Figure 2. Illustration of the sampling for Presence/Absence of *total coliforms* of the processing water in exporting mango packinghouses.

c. Microbiological quality on contact surfaces.

Regarding the results of sampling on contact surfaces of field boxes, packing boxes, fruit after washing, after hydrothermal, after hydrocooling, and fruit from packed boxes for *total coliform* and *total aerobic bacteria*; only a level of probable contamination higher than allowed in the Standard (NOM-127-SSA, 1994) was found in the fruit washing boxes as an indicator of poor hygiene practices. Therefore, it is suggested that these boxes should be washed and disinfected more frequently. It was observed that as the packing process progresses from the reception to fruit packing, the bacterial load decreases with the hydrocooling treatment and that good hygiene and handling practices are carried out during packaging to prevent contamination.

Table 3. ***Total aerobic bacteria*** **and** ***total coliforms*** **on contact surfaces in exporting mango packinghouses.**

	Initial (1)		Middle (7)		End of cycle (14)	
Treatment	*Total aerobic bacteria*	*Total coliforms*	*Total aerobic bacteria*	*Total coliforms*	*Total aerobic bacteria*	*Total coliforms*
Washing boxes	10^2	-	10^2	-	10^3	-
Fruit from washing boxes	10^2	-	10^2	-	10^2	-
Hydrothermal boxes	10^2	-	10^2	-	10^2	-
Fruit from hydrothermal boxes	10^2	-	10^2	-	10^2	-
Hydrocooling boxes	-	-	-	-	-	-
Fruit from hydrocooling boxes	-	-	-	-	-	-
Packed fruits	-	-	-	-	-	-
NOM-127-SSA	< 200 Col/ml	-	< 200 Col/ml	-	< 200 Col/ml	-

d. Fruit quality.

Practically no significant differences were detected in the organoleptic quality of the mango fruit obtained from hydrothermal tanks (T1), hydrocooling tanks (T2), or packed boxes (T3) in any of the stages of the complete sampling cycle (Figure 3), except for the external appearance at the end of cycle 1, where after seven days of cooling the fruits of T2 showed a better appearance than the fruits of T1 and T3. However, at consumption, the fruits of T3 (from boxes already packed), showed the best external appearance.

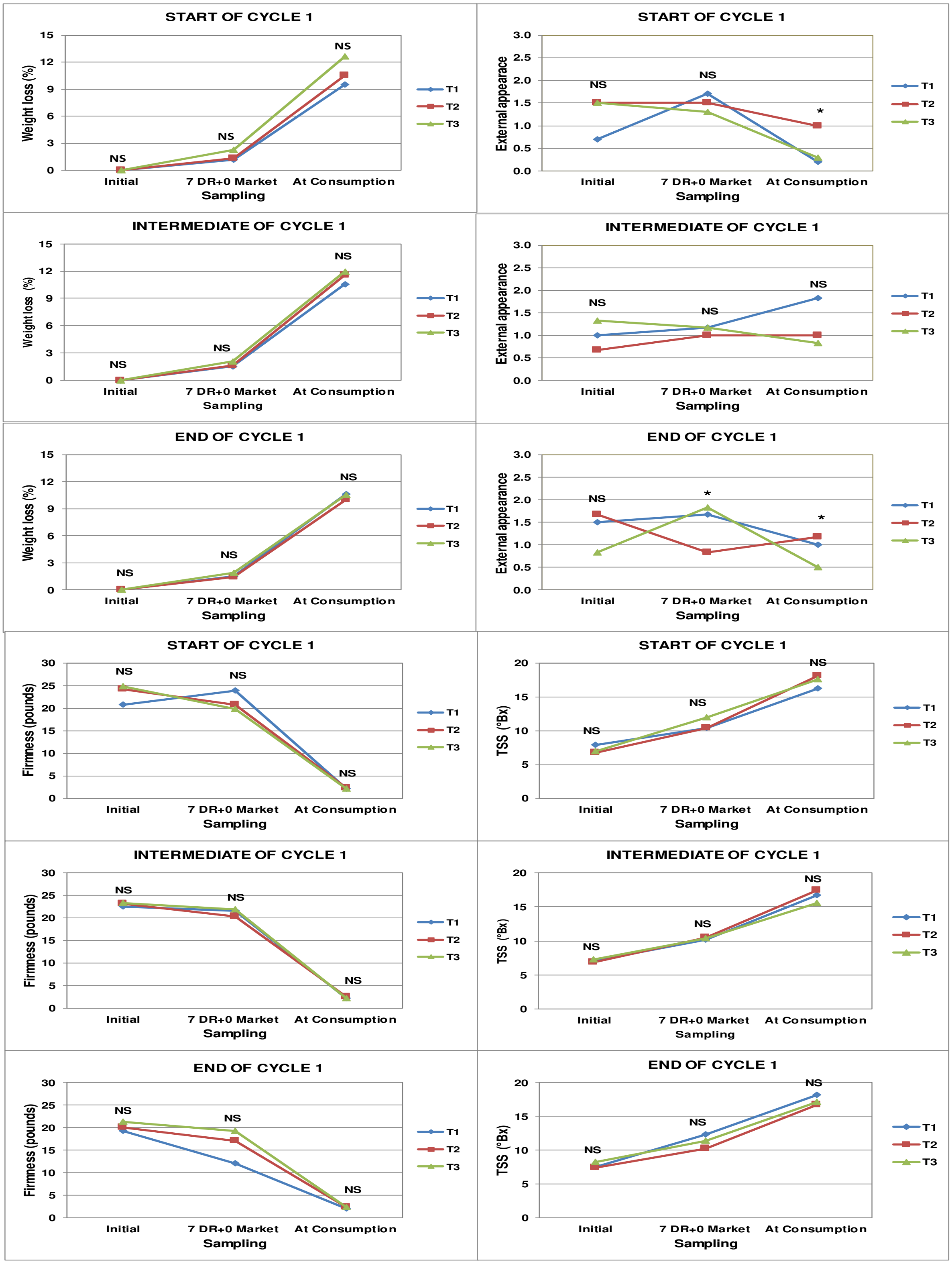


Figure 3. Organoleptic characteristics of sampled fruits from hydrothermal tanks (T1), hydrocooling tanks (T2) or packed boxes (T3) during a complete sampling cycle.

2. Scanning Packinghouse 2. Tommy Atkins.

a. Physical-Chemical quality of the processing water.

It was corroborated that the physical-chemical variable most affected was the turbidity since it far exceeded the critical value of the Standard (Table 4). The source water was the only one that was below the critical level of the Standard NMX-127 SSA (1994). However, it was observed in the processing water for fruit washing that at the end of a wash cycle of 1,200 boxes the turbidity value exceeded 12 times the critical value of the Standard (< 5). In addition, in water from hydrothermal tanks with 3, 5, or 7 days of use (hydrothermal 1, hydrothermal 2 and hydrothermal 3 tanks, respectively), a tendency to increase the turbidity value was observed as the number of days of use increased reaching almost five times the critical value of the Standard. As it is known, the time of hydrothermal treatment quarantine reaches up to 110 min maximum (depending on the size of the fruit), if the degree of turbidity is huge, the contamination potential risk of the fruits is too high. So, care must be taken to avoid the degree of turbidity not to exceed the limits established by the Standard. On the other hand, water from the hydrocooling tank with a week of use already showed a degree of turbidity twice the standard allowed by the Standard. In this area, measures must be taken to prevent the degree of turbidity from exceeding the limits established by the Standard.

Table 4. Physical-chemical composition of processing water in exporting mango packinghouses during a scanning.

Treatment	pH	Turbidity (UNT)	Total hardness (mg/L)	E. C. (dS m^{-1})	Sulfates (mg/L)	Chloride (mg/L)
Source	6.9	1.7	83.3	0.3	3.3	29.0
Initial washing	6.9	6.7	82.4	0.5	8.8	70.6
Final washing	6.0	60.7	149.0	0.2	36.2	87.6
Hydrothermal 1	7.7	14.3	76.8	0.4	13.9	28.8
Hydrothermal 2	7.6	18.7	76.1	0.4	19.8	27.4
Hydrothermal 3	7.6	38.3	85.5	0.4	43.3	30.2
Hydrocooling	7.3	11.3	73.1	0.3	41.2	25.1
Critical values	6.5 - 8.5	< 5.0	500.0	> 2.0	400.0	250.0

Figure 4 illustrates the degree of turbidity of the processing water in the different stages of handling for exporting mango fruit. It is notorious how the degree of turbidity in the final washing and in the hydrothermal tanks was increased as more treatment time elapsed. The suggestion is to avoid reaching levels that far exceed the critical level established by the Standard. The recommendation is to do more frequent changes of the processing water, in washing, hydrothermal and hydrocooling.

Figure 4. Illustration of the turbidity of the processing water in exporting mango packinghouses.

b. Microbiological quality of processing water.

Regarding the microbiological quality of the processing water during the scanning, the degree of water turbidity was reflected in the presence/absence of *total coliforms* since only the source water was free of these indicators of contamination (Table 5). The theory is confirmed that water with a higher degree of turbidity due to suspended particles such as soil and organic matter, is more prone to present possible contamination because the UV rays do not penetrate to the bottom of hydrothermal and hydrocooling tanks and do not allow to exert the microcida action.

Table 5. Presence/Absence of *total coliforms* in processing water in exporting mango packinghouses.

Treatment	*Total coliforms*
Source	-
Initial washing	+
Final washing	+
Hydrothermal 1 (3 d)	+
Hydrothermal 2 (5 d)	+
Hydrothermal 3 (7 d)	+
Hydrocooling (7 d)	+

(+) Presence (-) Absence

Figure 5 shows the results of the tests for the presence/absence of *total coliforms.* It is noted that only the source water sample was negative for the presence of *total coliforms*, and in this case even the hydrocooling water was positive for these microorganisms. This is because this water did not have any level of chlorination, for which it is recommended and under confirmation that a chlorine concentration of 20 to 50 ppm can prevent the presence of *total coliforms*.

Figure 5. Illustration of the result of the sampling for Presence/Absence of *total coliforms* of the processing water in exporting mango packinghouses.

c. Microbiological quality in contact surfaces.

The results found showed the high degree of potential contamination that the contact surfaces represent, because the high population of *total aerobic bacteria. Total aerobic bacteria* reflect the exposure of the sample to contamination in general, as well as, the existence of favorable conditions for the multiplication of pathogen microorganisms. In addition, the high presence of organic matter in field boxes, hydrothermal boxes, hydrocooling boxes, bands, and benches (Table 6) made *total aerobic bacteria* were equal to or greater than 10^3 when Standard NOM-127-SSA indicated levels < 200 Col/ml. The most critical point was detected in field boxes where the levels reached 10^6 Col/ml.

Table 6. *Total aerobic bacteria* and *total coliforms* on contact surfaces in exporting mango packinghouses.

	Initial	
Treatment	*Total aerobic bacteria*	*Total coliforms*
Field boxes	10^6	-
Fruit from field boxes	10^5	-
Hydrothermal 1 boxes	10^3	-
Fruit from hydrothermal 1 boxes	10^2	-
Hydrothermal 3 boxes	10^2	-
Fruit from hydrothermal 3 boxes	10^2	-
Hydrocooling boxes	10^3	-
Fruit from hydrocooling	10^2	-
Bands	10^3	-
Benches	10^3	-
Packed fruit	10^2	-
NOM-127-SSA	< 200 Col/ml	-

Figure 6 shows a field box with evident signs of lack of hygiene and even the fruits sampled from that box reached a very high level of *total aerobic bacteria* (10^5 Col/ml), which indicates a very high probability of microbiological contamination. It is essential that the exporting mango packinghouses implement a process of washing and sanitizing field boxes, otherwise, the probability of contamination with pathogenic microorganisms rises.

Figure 6. Illustration of the lack of hygiene of field boxes and their repercussions in the presence of *total aerobic bacteria*.

3. Scanning packinghouse 3. Kent.

a. Physical-chemical quality of processing water.

The results were very similar to those observed in packinghouse 2. The physical-chemical variable most affected was turbidity, since it far exceeded the critical value of the Standard (Table 7). The source water was the only one below the critical level. However, it was observed in the processing water for fruit washing that at the end of a wash cycle of 1,200 boxes the turbidity value exceeded by more than 50 times the critical value of the Standard and the pH became very acidic. The reason for this difference may be that in this packinghouse, they use peroxyacetic acid as a disinfectant, but the same mistake is made in washing too many boxes with the same water. In addition, in hydrothermal tanks water with 5, 9 or 19 baskets of use (hydrothermal 1, hydrothermal 2 and hydrothermal 3, respectively), a tendency to increase the turbidity value was observed as the number of processed baskets increased, to exceed four times the critical value of the Standard. As it is known, the hydrothermal treatment time lasts up to 110 min (depending on the size of the fruit), if the degree of turbidity is very high, the potential risk of contamination of the fruits is too high. The suggestion is to avoid reaching levels that far exceed the critical level established by the Standard. The recommendation is to do more frequent changes of the processing water, in washing, hydrothermal and hydrocooling. For this packinghouse, the hydrocooling water was still within the tolerable limits. On the other hand, another variable out of range was the total hardness of the water, which increased significantly in the final and hydrothermal washing stage 3. This may be influenced by the water source, the use of peroxyacetic acid and the accumulation of salts of magnesium and calcium.

Table 7. Physical-chemical composition of the processing water in exporting mango packinghouses during a scanning.

Treatment	pH	Turbidity (UNT)	Total hardness (mg/L)	E. C. (dS m^{-1})	Sulfates (mg/L)	Chloride (mg/L)
Source	7.8	1.0	165.7	1.0	3.6	113.2
Initial washing	6.9	9.7	427.9	1.0	11.4	114.6
Final washing	5.8	265.3	959.3	1.2	78.7	112.1
Hydrothermal 1	7.7	8.7	459.6	1.1	11.4	150.5
Hydrothermal 2	7.5	13.7	411.5	1.1	16.4	119.8
Hydrothermal 3	7.7	20.0	632.7	1.3	18.1	184.8
Hydrocooling	7.9	4.3	215.2	1.1	16.8	100.2
Critical values	6.5 - 8.5	< 5.0	500.0	> 2.0	400.0	250.0

Figure 8 illustrates the degree of turbidity of the processing water in the different stages of handling the mango fruit for export. It is notorious how the degree of turbidity in the final washing and in the hydrothermal tanks increases as more treatment time elapses. The suggestion is to avoid reaching levels that far exceed the critical level established by the Standard. Therefore, more frequent changes must be made to the processing water in both washing and hydrothermal.

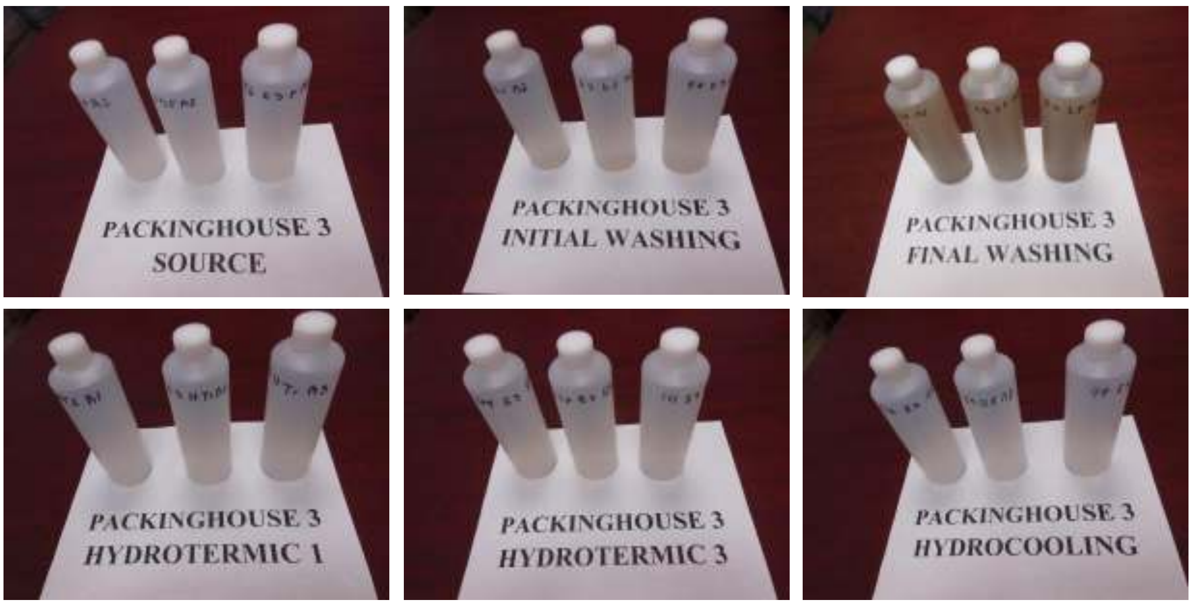


Figure 8. Illustration of the turbidity of the processing water in exporting mango packinghouses.

b. Microbiological quality of processing water.

Regarding the microbiological quality of the process water during the scanning in the packinghouse 3, it was found that in the stages where microbiological control is used (peroxyacetic acid in washing and chlorine in hydrocooling) the presence of *total coliforms* was not detected (Table 8). However, in hydrothermal tanks, where there was no control, the presence of *total coliforms* was found. It confirms the previously described, that it is essential to have the control point in the hydrocooling tank with a concentration of 20 to 50 ppm of chlorine.

Figure 9 shows the results of the tests for the presence/absence of total coliforms. It is noted that only water samples that had no control were positive for the presence of *total coliforms*, confirming that both peroxyacetic acid (20 ppm) and chlorine can prevent the presence of these microorganisms.

Table 8. Presence/Absence of *total coliforms* in processing water in exporting mango packinghouses.

Treatment	*Total coliforms*
Source	-
Initial washing	-
Final washing	-
Hydrothermal 1 (5 baskets)	+
Hydrothermal 3 (19 baskets)	+
Hydrocooling	-

(+) Presence (-) Absence

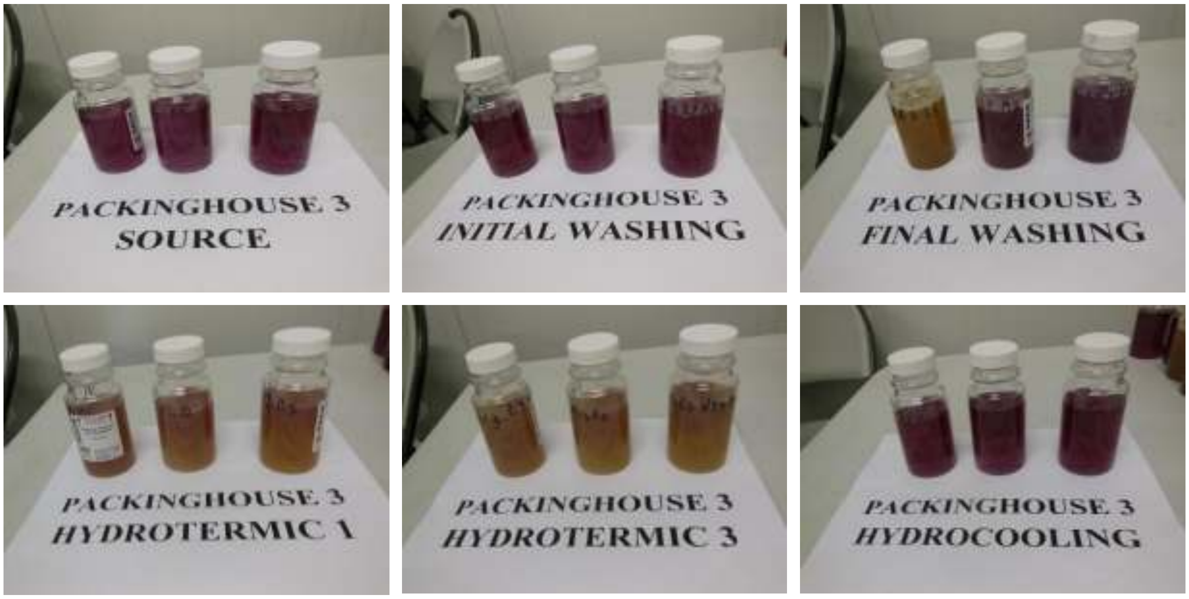


Figure 9. Illustration of the result of the sampling for Presence/Absence of *total coliforms* of the processing water in exporting mango packinghouses.

c. Microbiological quality on contact surfaces.

For this packinghouse, the conditions were less severe than in packinghouse 2. However, the results showed that the field boxes are still a critical point to control and avoid potential contamination since the levels reached 10^5 Col/ml, exceeding the values of the Standard (Table 9).

Table 9. *Total aerobic bacteria* and *total coliforms* on contact surfaces in exporting mango balers packinghouses.

	Initial	
Treatment	*Total aerobic Bacteria*	*Total coliforms*
Field boxes	10^5	-
Fruit from field boxes	10^4	-
Hydrothermal 1 boxes	10^2	-
Fruit from Hydrothermal 1 boxes	10^2	-
Hydrothermal 3 boxes	10^2	-
Fruit from Hydrothermal 3 boxes	10^2	-
Hydrocooling boxes	10^2	-
Fruit hydrocooling boxes	10^2	-
Bands	10^2	-
Benches	10^3	-
Packed fruit	10^2	-
NOM-127-SSA	< 200 Col/ml	-

Figure 10 shows field boxes presenting a lack of hygiene practices and even the fruits sampled from these boxes reached a high level of *total aerobic bacteria* (10^4 Col/ml) that indicate a high probability of microbiological contamination. It is essential that exporting mango packinghouses implement a process of washing and sanitizing field boxes, otherwise, the probability of contamination of pathogenic microorganisms increases.

Figure 10. Illustration of the lack of hygiene of field boxes and their repercussions in the presence of *total aerobic bacteria*.

4. Preventing contamination in processing water.

a. Effectiveness of chloride as a disinfectant.

One of the first questions was to determine the minimum concentration of chlorine to control the presence of *total aerobic bacteria* and *total coliforms*. In Table 10, it was observed that the control without chlorine presented a population of 10^3 of *total aerobic bacteria,* and positive for total coliforms. At 10 ppm, it was still possible to detect *total aerobic bacteria* but not *total coliforms*. To maintain a safety range, it was determined the minimum chlorination level should be 20 ppm.

Table 10. *Total aerobic bacteria* and *total coliforms* in water and fruit at different chlorine concentrations.

	Initial	
Treatment	***Total aerobic Bacteria***	***Total coliforms***
Control	**10^3**	**+**
10 ppm	**10^2**	**-**
20 ppm	**-**	**-**
30 ppm	**-**	**-**
40 ppm	**-**	**-**
50 ppm	**-**	**-**
NOM-127-SSA	**< 200 Col/ml**	**-**

(+) Presence (-) Absence

Figure 11 illustrates the presence of *total aerobic bacteria* and *total coliforms* in washing water or fruit surface treated with different concentrations of chlorine. It was observed the control without chlorine has presence of both microorganism, but from 20 ppm these are controlled.

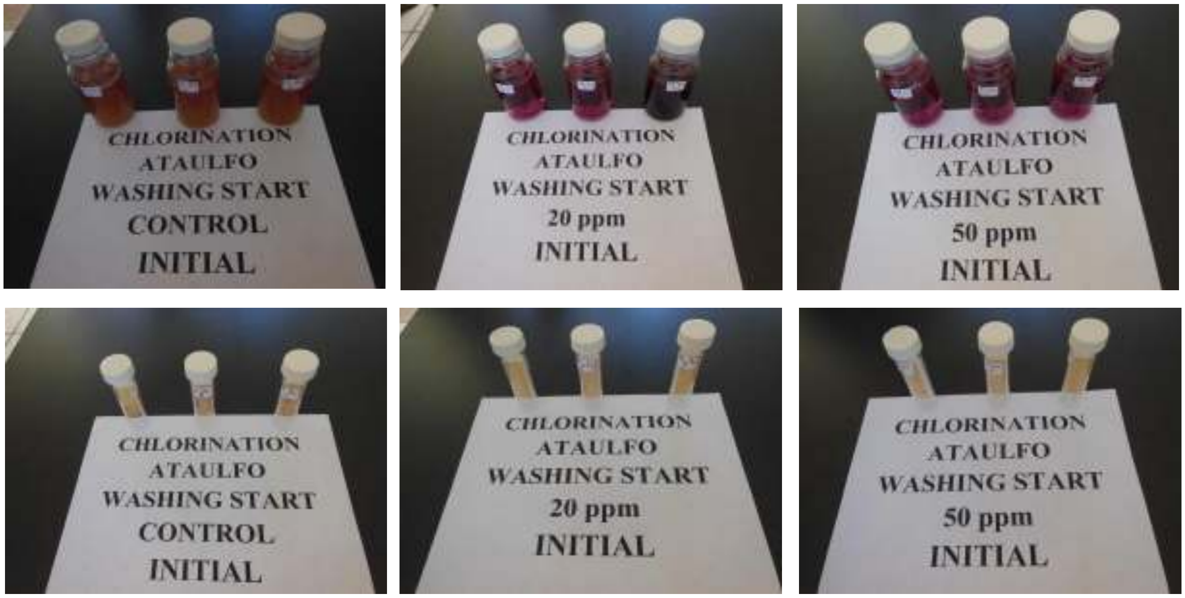


Figure 11. Illustration of the effectiveness of chlorine as a disinfectant in water and/or surface of fruits washed at different concentrations.

b. Determination of chlorine injury.

The next question to answer was whether high chlorine concentrations could affect fruit quality characteristics. In Table 11, it was observed the control without chlorine had a population of 10^3 *total aerobic bacteria* and positive for *total coliforms*. From 50 ppm of chlorine, the presence of *total aerobic bacteria* and *total coliforms* was completely inhibited, being consistent with the previous activity where it was detected that from 20 ppm is a minimum level of chlorination to maintain a safe range in the control of pathogenic microorganisms. Similarly, Figure 12 illustrates the control of chlorine concentrations, where any of them inhibited the presence of *total aerobic bacteria* and *total coliforms*.

Table 11. *Total aerobic bacteria* and *total coliforms* in water and fruit at different chlorine concentrations.

	Initial	
Treatment	***Total aerobic Bacteria***	***Total Coliforms***
Control	**10^3**	**+**
50 ppm	**-**	**-**
100 ppm	**-**	**-**
150 ppm	**-**	**-**
200 ppm	**-**	**-**
NOM-127-SSA	**< 200 Col/ml**	**-**

(+) Presence (-) Absence

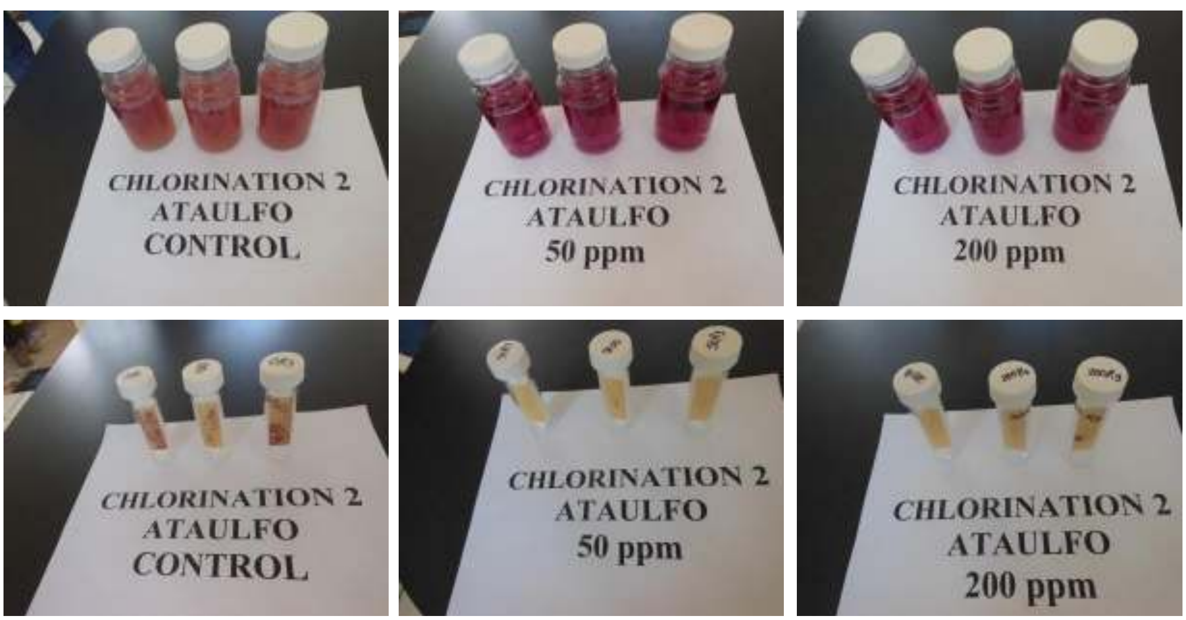


Figure 12. Illustration of the effectiveness of chlorine as a disinfectant in water and/or surface of fruits washed at different concentrations.

The answer to the question of whether high chlorine concentrations affected fruit quality characteristics is observed in Figure 13. It was observed that even at 200 ppm none of the quality characteristics was affected since no significant differences were detected among treatments for none of the variables. This shows that initial concentrations of 200 ppm of chlorine did not affect the quality of the fruits, which was the fear of the packers (Figure 14).

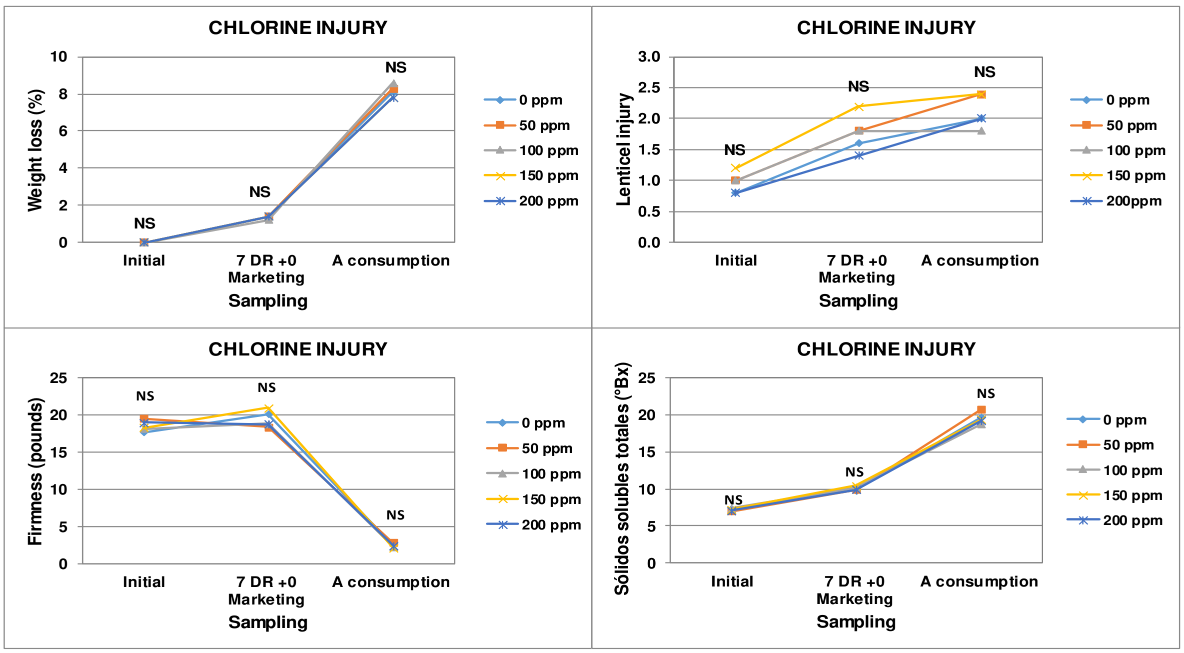


Figure 13. Illustration of the effect of chlorine concentrations on the fruit quality characteristics of Ataulfo variety.

Figure 14. Illustration of the effect of chlorine concentrations on the fruit quality characteristics of Ataulfo variety.

c. Effectiveness of chlorine concentrations on washing water with prolonged use.

Another question of the packers was to know when chlorine concentrations in washing water are reduced. In Figure 15, in a washing cycle with clean water at an initial concentration of 200 ppm, it was observed that due to the impurities, dirt and organic matter contained in the fruits, the chlorine concentration drops to almost zero at the end of the 600 washing boxes of fruits. It would indicate that the ideal is to make washing cycles of 600 boxes changing the water and bringing back to an initial concentration of 200 ppm of chlorine.

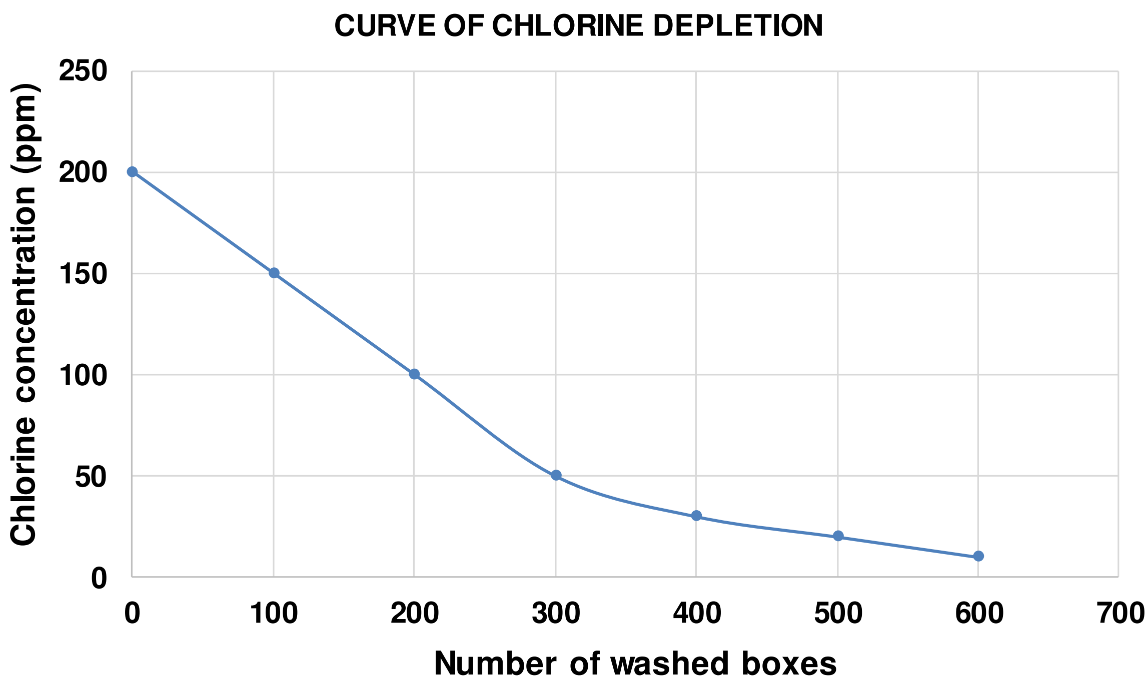


Figure 15. Curve of chlorine depletion according to the number of washed boxes

However, in mango packinghouses up to five trucks arrive at the time of reception. For those who have only one washing line it is complicated to make water changes and new concentration of chlorine, since washing a truck with 600 boxes of fruit takes them on the average 45 min to one hour, and the change of water means at least 30-40 min. Therefore, the next question to clarify was if it is possible to use the same washing water only leading to an initial chlorination of 200 ppm. The physical-chemical results of the washing water with prolonged use (Table 12) showed that as the water is used for a longer time, all the variables are altered: the pH becomes acidic; hardness, electrical conductivity, sulfates and chlorine is increased although at levels still within the Standard. The only variable that far exceeds the norm was the turbidity of the water, which increased as the number of washing cycles increased (Figure 16).

Table 12. Physical-chemical analysis of washing water with prolonged use.

Washing cycles	pH	Turbidity (UNT)	Total hardness (mg/L)	C.E. (dS m^{-1})	Sulfates (mg/L)	Chloride (mg/L)
First	7.0	21.7	142.5	0.3	9.7	58.1
Second	7.2	19.7	169.1	0.5	20.2	62.3
Third	7.0	28.7	242.6	0.6	39.1	91.7
Fourth	6.8	47.7	294.2	0.7	49.2	123.6
Fifth	6.6	46.7	386.6	0.8	56.0	160.5
NOM-127-SSA	6.5 - 8.5	< 5.0	500.0	> 2.0	400.0	250.0

Figure 16. Illustration of the turbidity of the washing water with prolonged use.

On the other hand, the fruit quality characteristics of Kent variety showed affectation of all the variables (Figure 17), especially external damage, which as its use intensified, it was reflected in greater lenticel damage (Figure 18).

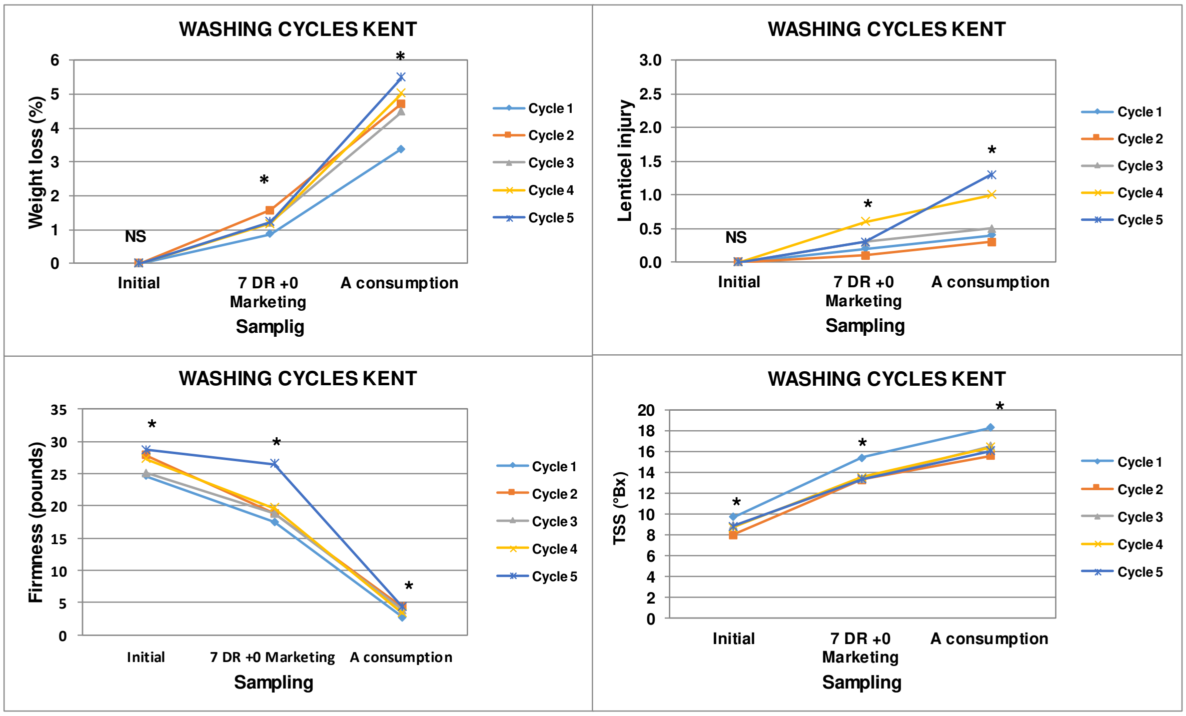


Figure 17. Illustration of the effect of chlorinated water of prolonged use on the fruit quality characteristics of Kent variety.

Figure 18. Illustration of the effect of chlorinated water of prolonged use on the external appearance of fruits of Kent variety.

It was concluded the water of prolonged use with 200 ppm of initial chlorine could be used up to a maximum of three cycles. Although, it is advisable to change the water at the end of each cycle of washing 600 boxes.

5. Diagnosis of 'Black cut' and 'Spongy Tissue' in Ataulfo variety

At the request of Dr. Leonardo Ortega, Research Director of the NMB, an additional activity was included to determine only in 'Ataulfo' whether the ripening degree at harvest and the duration of refrigerated storage influenced the manifestation of 'Black Cut' and 'Spongy Tissue'. The hypothesis to be tested was that partially ripened fruits and a longer duration of refrigerated storage would have a greater presence of both disorders. Brecht (2017, Com. Pers.) states that fruits of 'Ataulfo' stored for three weeks at 12 ± 1°C in normal air atmosphere presented approximately 30% of fruits with 'Black Cut', while those stored under the same temperature, but in modified atmospheres completely prevented this disorder. However, the results found here showed that practically the percentage of fruits with 'Black Cut' was insignificant because it did not reach even 3% of the total sample observed (450 partially ripened fruits and 450 ripened fruits) since during the date of start of season (May 3rd) the presence of this anomaly was zero. The intermediate date of June 7th was the one that showed a relatively greater percentage of 'Black Cut' since at maturity of consumption after three weeks of storage, it was found up to 15% of fruits with this disorder without there being any difference for the initial degree of maturity (Table 13). Already on the third date of June 27th, the presence of 'Black Cut' was detected in only 5% of the fruits after 1 and 2 weeks of storage. Therefore, it was concluded that neither the maturity factor nor the storage time were decisive for the presence of fruits with 'Black Cut' in Ataulfo variety. The presence of this anomaly was even lower for Spongy Tissue, which occurred during harvest 2 and 3 and maximum in 5%, but only in partially ripened fruits. It seems that both disorders may be influenced by other factors, perhaps related to nutrition and probably imbalances of Nitrogen and/or Calcium.

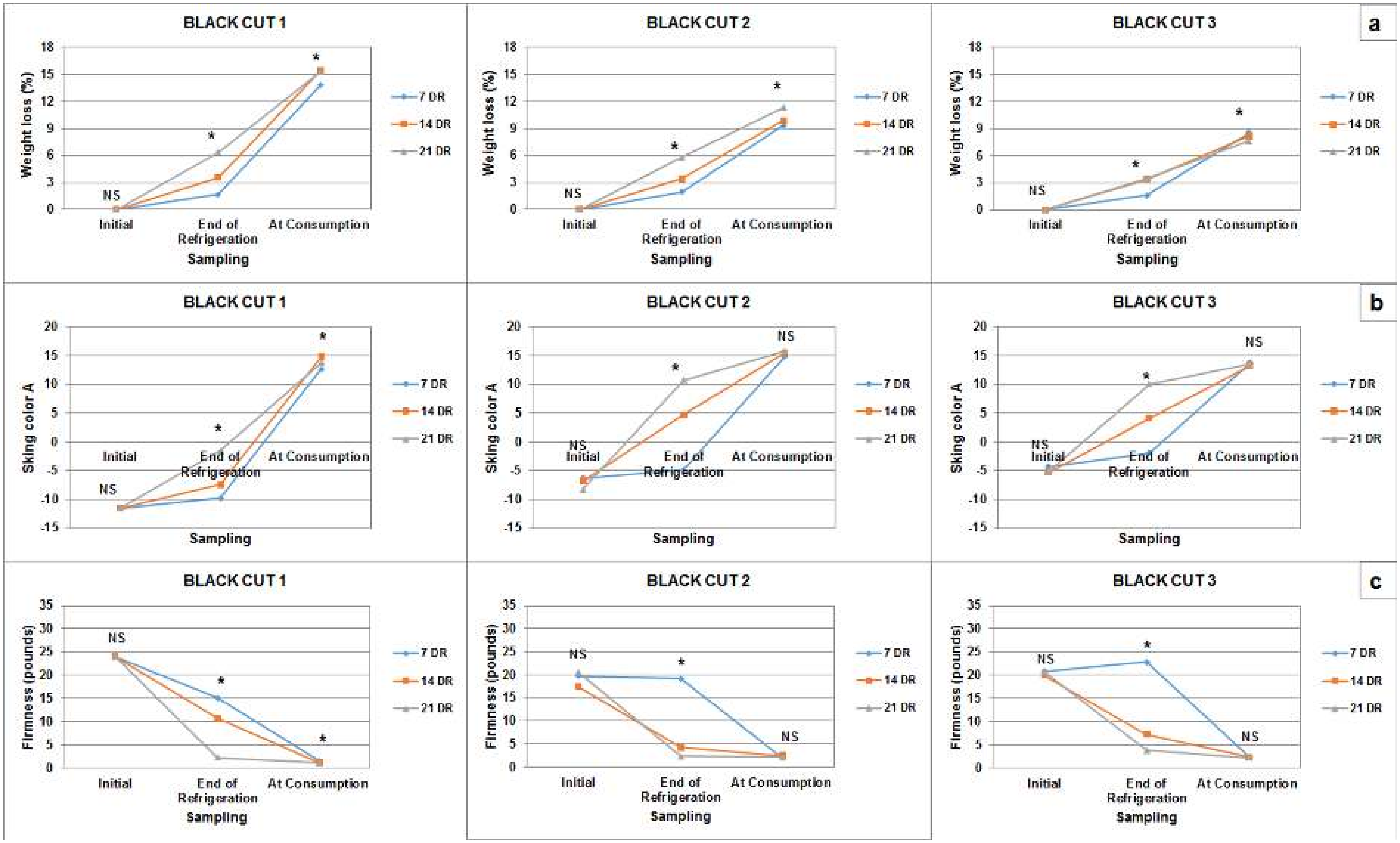
Table 13. Percentage of fruits with 'Black Cut' or 'Spongy Tissue' in Ataulfo fruits harvested at two ripening degree and stored up to three weeks at 12 ± 1°C.

<table>
<tr><th colspan="7">Percentage of fruit with 'Black Cut' (BC) or Spongy Tissue (ST)</th></tr>
<tr><th>Activity</th><th>Ripening degree</th><th>Initial</th><th>Week 1</th><th>Week 2</th><th>Week 3</th><th>Harvesting date</th></tr>
<tr><td rowspan="2">Black Cut1</td><td>Partial ripe</td><td>0</td><td>0</td><td>0</td><td>0</td><td rowspan="2">May 3, 2016</td></tr>
<tr><td>Ripe</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="2">Black Cut2</td><td>Partial ripe</td><td>0</td><td>0</td><td>ST= 5%</td><td>BC=15%
ST=5%</td><td rowspan="2">June 7, 2016</td></tr>
<tr><td>Ripe</td><td>0</td><td>0</td><td>BC=10%</td><td>BC=15%</td></tr>
<tr><td rowspan="2">Black Cut3</td><td>Partial ripe</td><td>0</td><td>BC=5%</td><td>BC=5%
ST=5%</td><td>0</td><td rowspan="2">June 27, 2016</td></tr>
<tr><td>Ripe</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>

Figure 19 illustrates the effect of ripening degree and storage time on the internal color of 'Ataulfo' fruit at consumption. It was possible to observe that the presence of ‘Black Cut' and 'Spongy Tissue' was minimal.

Figure 19. Effect of ripening degree and storage time on the presence of 'Black Cut' or 'Spongy Tissue' in fruits of 'Ataulfo' at consumption stage.

On the other hand, results of this assay allow us to confirm the effect of prolonged storage under conventional refrigeration on main quality variables (Figure 20). At the end of refrigeration for one, two, or three weeks, the weight loss increased, the skin color and pulp firmness decreased, and the development of total soluble solids and pulp color was accelerated.


a
BLACK CUT 1
BLACK CUT 2
BLACK CUT 3
Weight loss (%)
18
15
12
9
6
3
0
NS
*
Initial
End of Refrigeration
At Consumption
Sampling
7 DR
14 DR
21 DR
b
BLACK CUT 1
BLACK CUT 2
BLACK CUT 3
Sking color A
20
15
10
5
0
-5
-10
-15
NS
*
Initial
End of Refrigeration
At Consumption
Sampling
7 DR
14 DR
21 DR
c
BLACK CUT 1
BLACK CUT 2
BLACK CUT 3
Firmness (pounds)
35
30
25
20
15
10
5
0
NS
*
Initial
End of Refrigeration
At Consumption
Sampling
7 DR
14 DR
21 DR

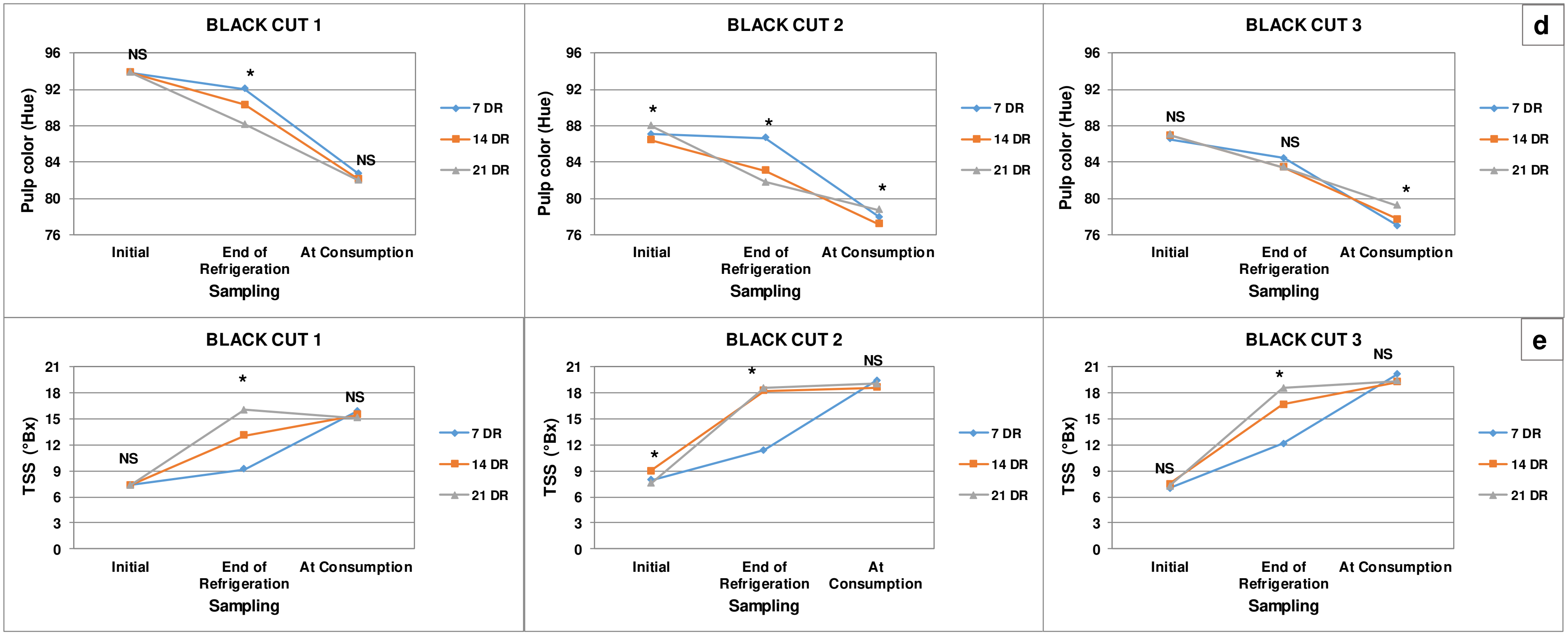


Figure 20. Effect of ripening degree and refrigerated storage time (12 ± 1°C) on weight loss (a), skin color (b), firmness (c), pulp color (d) and total soluble solids (e) in 'Ataulfo' fruits.

CONCLUSIONS

- For water quality, turbidity was the most affected variable, which increased according the prolonged use favoring microbial growth. In QHWT tanks, it is suggested to change the water no longer than 14 baskets by cycle.
- The water with prolonged use in washing or hydrothermal tanks presented *total coliforms* and *total aerobic bacteria* indicating microbial contamination risk. However, if the water in the hydrocooling tank is maintained at 20-50 ppm chlorine, the pathogenic microorganisms are controlled.
- The most critical point for fruit contamination in contact surfaces was field boxes, which should be washed and disinfected before returning to the field.
- No organoleptic differences were found among fruit coming from hydrothermal or hydrocooling tanks nor packed boxes.
- Concerning to the use of chlorine as a disinfectant, it was effective at 20 ppm to eliminate *total coliforms* and *total aerobic bacteria* without affecting fruit quality even at initial concentration of 200 ppm.
- The initial chlorine concentration should be 200 ppm, since it brings down until 10 ppm at the end of a washing cycle with 600 boxes of mango fruit.
- Chlorine in water with prolonged use should be used no more than three cycles, but the recommendation is to change the water of washing tanks every cycle of 600 boxes.
- The presence of ‘Black Cut' and ‘Spongy Tissue’ was practically inappreciable in the three surveys done during the 2016 harvest season. Neither the ripening degree nor the duration of refrigerated storage affected both physiological disorders.
- Results of this experiment allow us to confirm the effect of long-term conventional refrigeration on main quality variables. At the end of refrigeration for one, two, or three weeks, the weight loss increased, the skin color and pulp firmness decreased, and the development of total soluble solids and pulp color was accelerated.

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