# QUARANTINE COLD-STORAGE TREATMENT ON SATSUMA MANDARIN *CITRUS UNSHIU* FOR EXPORT TO JAPAN

*Eng. Agr. Ezequiel F. Quenta*

*Quarantine Section Especialist*

*Plant Health Division*

*SENASA - Perú*

# EXPERT CONSULTATION ON COLD TREATMENT IPPC-FAO
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***Eng. Agr. Ezequiel F. Quenta***

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# TITLE

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# Introduction

- The main problem of the Peruvian fruit represents the presence of the Mediterranean fruit fly *Ceratitis capitata* mainly in citrus production areas, located in the central coast of Peru.

- Since 2006 the export of citrus from Peru to the U.S., is made with cold quarantine treatment, with temperatures of 1.11 and 1.67° C for 15 and 17 days respectively, but Peruvian exporters report that at this temperature the fruit sustains damage to its quality, reason why is proposed using temperatures higher than 2.1 and 3.1° C at the core of the fruit.

- With this purpose the Ministry of Agriculture of Peru through SENASA submitted a Work Plan for the Ministry of Agriculture Forestry and Fisheries of Japan MAFF to lift the ban on Peruvian citrus and access this important market
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# Objective

To prove that quarantine cold treatment is effective as a mitigating measure to avoid the survival of eggs and/or larvae of the fruit fly (*Ceratitis capitata* Wiedemann) in Satsuma variety mandarin *Citrus unshiu* to access the Japanese market

# Especific objectives

**Basic disinfestations Test:**

- Research on the development of the mediterranean fruit fly in satsuma mandarin.
- Research de most cold-tolerant development stage on satsuma mandarin
- Small-scale disinfestation test to determine the conditions of the treatment

**Applied Disinfestation Test:**

- Large-scale disinfestation test with most cold-tolerant stage to confirm the total eradication of the mediterranean fruit fly in Satsuma mandarin
- Quality Test to evaluate the effect of the cold storage on the quality of the satsuma mandarin.

# Materials and Methods

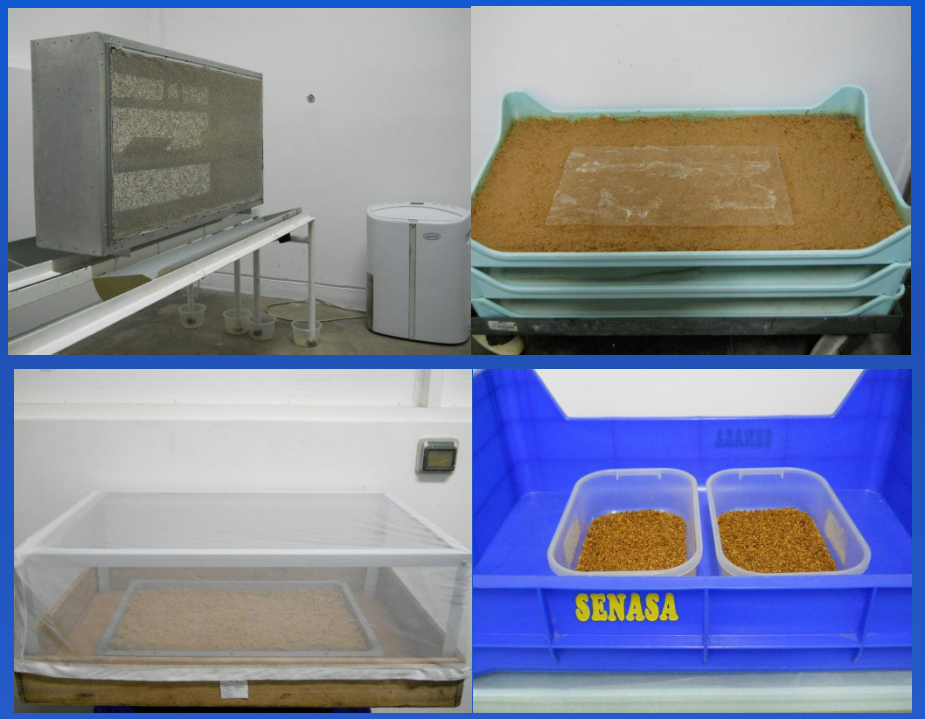
**Test Insect**

- ✓ Mediterranean fruit fly *Ceratitis capitata* Wiedemann.
- ✓ Host of origin: from *Terminalia catappa* Combretaceae L.
- ✓ Place: Casma Valley, Central Coast, Department Ancash
- ✓ Generation F4

# Materials and Methods

**Rearing method of *C. capitata***

- ✓ Cage of adults
- ✓ Artificial diet for larvae
- ✓ Colect of larvae
- ✓ Maturation of pupae
- ✓ Environmental conditions: 25º ± 1º C and 60 ± 5% HR; Light / Darkness 10:14



# Materials and Methods

**Test fruit**

- ✓ Satsuma mandarin harvested in Huaral – Lima and Chincha Ica.
- ✓ Size 1X and 2 X (68 to 78 mm in diameter, weight 125 to 150 g, with a uniform maturation grade.

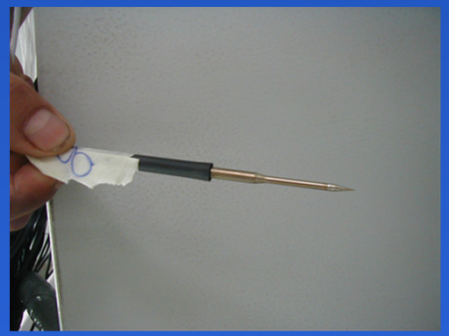
# Materials and Methods
# Test Facilities

- Quarantine Treatment Center - SENASA
- Av. La Molina 1915, La Molina District, Lima Department - Perú

# Materials and Methods
# Test Facilities

## Isothermal Chambers for Treatment

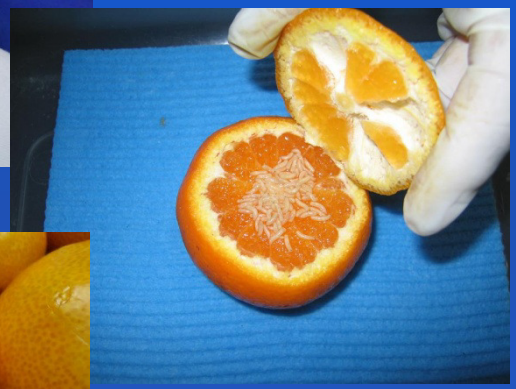
- ✓ Two Chambers 7m3 each one
- ✓ PLC Unitronics Vision 120 ™ each one
- ✓ 12 Insertion sensor JUMO® each one

# Materials and Methods

**Fruit Infestations**

- Method of inoculation: Cap method
- Artificial inoculation of eggs.
- Artificial inoculatión of larvae 3rd instar



# Materials and Methods

**Cold Treatment:**

- ✓ Sensor calibration water-ice immersion method
- ✓ Temperature: 2.1 and 3.1º ± 0.5º C at core of the fruit.
- ✓ 10 sensor located in the fruit without infestation
- ✓ 2 environmental sensor of the chamber
- ✓ Load factor 13.3 and 16.7%


# Materials and Methods

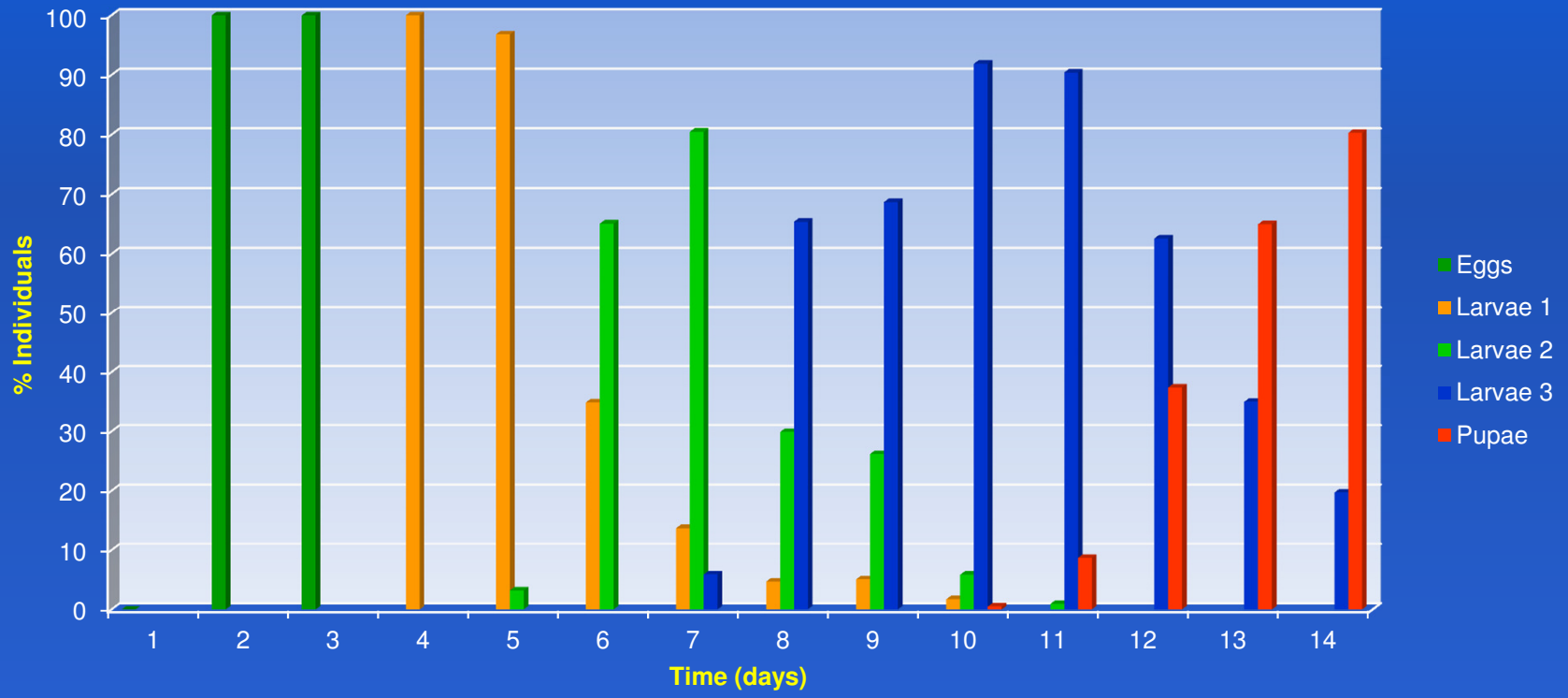
**Evaluation of Cold Treatment**

Fruit infested and fruit control

- For fruit with Eggs after 72 hours after treatment
- For young larvae after 48 hours after treatment
- For mature larvae after 24 hours after treatment

# Results: Development Immature Stages of Medfly

**Age composition of *C. capitata* in Satsuma mandarin *Citrus unshiu* at 24.8º C and 61.8% HR**



# Results: Tolerance Test

**Mortality of immature stages of the medfly *C. capitata*, stored a 2.1º C during several period of exposure - First Replication**

| Stage Insects | Exposure Period (days) | Number of fruits | Number off eggs inoculated | Live insects | Corrected mortality Abbot (%) |
|---|---|---|---|---|---|
| Eggs 20 hours | control | 20 | 2000 | 1414 | |
| | 2 | 20 | 2000 | 375 | 73.48 |
| | 4 | 20 | 2000 | 331 | 76.59 |
| | 6 | 20 | 2000 | 165 | 88.33 |
| | 8 | 20 | 2000 | 1 | 99.93 |
| | 10 | 20 | 2000 | 0 | 100.00 |
| | 12 | 20 | 2000 | 0 | 100.00 |
| | 14 | 20 | 2000 | 0 | 100.00 |
| Young Larvae L1 - L2 | control | 20 | 2000 | 1517 | |
| | 2 | 20 | 2000 | 1264 | 16.68 |
| | 4 | 20 | 2000 | 1271 | 16.22 |
| | 6 | 20 | 2000 | 289 | 80.95 |
| | 8 | 20 | 2000 | 75 | 95.06 |
| | 10 | 20 | 2000 | 8 | 99.47 |
| | 12 | 20 | 2000 | 0 | 100.00 |
| | 14 | 20 | 2000 | 0 | 100.00 |
| Mature Larvae L3 | control | 20 | 2000 | 1414 | |
| | 2 | 20 | 2000 | 1260 | 10.89 |
| | 4 | 20 | 2000 | 1035 | 26.80 |
| | 6 | 20 | 2000 | 365 | 74.19 |
| | 8 | 20 | 2000 | 68 | 95.19 |
| | 10 | 20 | 2000 | 12 | 99.15 |
| | 12 | 20 | 2000 | 10 | 99.29 |
| | 14 | 20 | 2000 | 0 | 100.00 |

**Mortality of immature stages of the medfly *C. capitata*, stored a 2.1º C during several period of exposure - Second Replication**

| Stage Insects | Exposure Period (days) | Number of fruits | Number off eggs inoculated | Live insects | Corrected mortality Abbot (%) |
|---|---|---|---|---|---|
| Eggs 20 hours | control | 20 | 2000 | 1348 | |
| | 2 | 20 | 2000 | 356 | 73.59 |
| | 4 | 20 | 2000 | 334 | 75.22 |
| | 6 | 20 | 2000 | 158 | 88.28 |
| | 8 | 20 | 2000 | 0 | 100.00 |
| | 10 | 20 | 2000 | 0 | 100.00 |
| | 12 | 20 | 2000 | 0 | 100.00 |
| | 14 | 20 | 2000 | 0 | 100.00 |
| Young Larvae L1 - L2 | control | 20 | 2000 | 1551 | |
| | 2 | 20 | 2000 | 1533 | 1.16 |
| | 4 | 20 | 2000 | 1309 | 15.60 |
| | 6 | 20 | 2000 | 458 | 70.47 |
| | 8 | 20 | 2000 | 76 | 95.10 |
| | 10 | 20 | 2000 | 6 | 99.61 |
| | 12 | 20 | 2000 | 2 | 99.87 |
| | 14 | 20 | 2000 | 0 | 100.00 |
| Mature Larvae L3 | control | 20 | 2000 | 1348 | |
| | 2 | 20 | 2000 | 1231 | 8.68 |
| | 4 | 20 | 2000 | 1206 | 10.53 |
| | 6 | 20 | 2000 | 448 | 66.77 |
| | 8 | 20 | 2000 | 115 | 91.47 |
| | 10 | 20 | 2000 | 7 | 99.48 |
| | 12 | 20 | 2000 | 0 | 100.00 |
| | 14 | 20 | 2000 | 1 | 99.93 |

# Results: Tolerance Test

| Tolerance Test: Lethal Dose (LD's) at 95% in days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Rep. | Stage | LD50 (95%CL) | | | LD90 (95%CL) | | | LD95 (95%CL) | | |
| | | **Days** | Lower | Upper | **Days** | Lower | Upper | **Days** | Lower | Upper |
| 1 | Egg | SC | | | SC | | | SC | | |
| | Young Larva | **5.14** | 3.98 | 5.83 | **7.02** | 6.16 | 9.06 | **7.67** | 6.67 | 10.74 |
| | Adult Larva | **4.98** | 4.33 | 5.48 | **7.36** | 6.7 | 8.4 | **8.23** | 7.4 | 9.72 |
| 2 | Egg | SC | | | SC | | | SC | | |
| | Young Larva | **5.23** | 5.15 | 5.3 | **7.26** | 7.15 | 7.38 | **7.97** | 7.83 | 8.13 |
| | Adult Larva | **5.53** | 4.91 | 5.99 | **7.63** | 7.07 | 8.5 | **8.36** | 7.66 | 9.6 |

# Conclusion: Tolerance Test

- Mature larvae (3rd instar) were more tolerant to cold, suggesting this stage of development to continue testing small-and large-scale
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# Results: Small-scale disinfestation Test at 2.1 ± 0.5º C

**Small-scale disinfestation Testing: Mortality of mature larvae (3rd stage) of *C. capitata* in Satsuma mandarin stored at a temperature of 2.1 ° C ± 0.5 ° C during several days of exposure.**

| Replication | Exposure Period Days | Nº fruits | Number of Insects per fruit | No. Total Estimated insects treated | Nº Survivors Total | Mortality Abbott (%) |
|---|---|---|---|---|---|---|
| 1 | Control | 42 | 100 | 4200 | 2786 | |
| | 6 | 40 | 100 | 4000 | 866 | 68.92 |
| | 8 | 40 | 100 | 4000 | 209 | 92.50 |
| | 10 | 40 | 100 | 4000 | 24 | 99.14 |
| | 12 | 40 | 100 | 4000 | 10 | 99.64 |
| | 14 | 40 | 100 | 4000 | 1 | 99.96 |
| | 16 | 40 | 100 | 4000 | 1 | 99.96 |
| | 18 | 40 | 100 | 4000 | 0 | 100.00 |
| 2 | Control | 42 | 100 | 4200 | 2578 | |
| | 6 | 40 | 100 | 4000 | 708 | 72.54 |
| | 8 | 40 | 100 | 4000 | 174 | 93.25 |
| | 10 | 40 | 100 | 4000 | 24 | 99.07 |
| | 12 | 40 | 100 | 4000 | 10 | 99.61 |
| | 14 | 40 | 100 | 4000 | 2 | 99.92 |
| | 16 | 40 | 100 | 4000 | 0 | 100.00 |
| | 18 | 40 | 100 | 4000 | 0 | 100.00 |

# Results: Small-scale disinfestation test at 3.1 ± 0.5º C

**Small scale disinfestation testing: Mortality of C. capitata in Satsuma mandarins stored at a temperature of 3.1 ° C ± 0.5 ° C during several days of exposure.**

| Replication | Exposition period Days | Nº fruits | Number of Insects per fruit | Nº Total Estimated insects treated | Nº Survivors Total | Mortality Abbott (%) |
|---|---|---|---|---|---|---|
| 1 | Control | 60 | 100 | 6000 | 3137 | |
| | 12 | 60 | 100 | 6000 | 13 | 99.59 |
| | 14 | 60 | 100 | 6000 | 9 | 99.71 |
| | 16 | 60 | 100 | 6000 | 9 | 99.71 |
| | 18 | 60 | 100 | 6000 | 6 | 99.81 |
| | 20 | 60 | 100 | 6000 | 1 | 99.97 |
| | 22 | 60 | 100 | 6000 | 0 | 100.00 |
| 2 | Control | 60 | 100 | 6000 | 3165 | |
| | 12 | 60 | 100 | 6000 | 16 | 99.49 |
| | 14 | 60 | 100 | 6000 | 10 | 99.68 |
| | 16 | 60 | 100 | 6000 | 9 | 99.72 |
| | 18 | 60 | 100 | 6000 | 4 | 99.87 |
| | 20 | 60 | 100 | 6000 | 0 | 100.00 |
| | 22 | 60 | 100 | 6000 | 0 | 100.00 |

# Conclusion: Small-scale disinfestation Test

- According to the results obtained with the small- scale test, we conclude that the treatment condition at 2.1 ± 0.5º C at the core of the fruit, in 18-days was enough to reach 100% of mortality the mature larvae (3rd instar) of *C. capitata* Wied.

- According to the results obtained in small-scale test concluded that the treatment condition of 3.1 ± 0.5º C with a period of 22 days was achieved the 100% of mortality of *C. capitata*, recommending this condition on large scale test.

# Results: Larger-scale disinfestation Test at 2.1± 0.5º C

**Larger scale disinfestation test: Mortality of mature larvae (3rd instar) of *C. capitata*, inside Satsuma mandarin at 2.1 º C on average**

| Replication | Control Fruits | | Treated Fruits | | | |
|---|---|---|---|---|---|---|
| | Nº of Fruits | Living insects | Nº of Fuits | Estimated Nº of treated insects | Total Nº of survivors | Mortality % |
| 1 | 75 | 3827 | 225 | 11481 | 0 | 100 |
| 2 | 80 | 5053 | 240 | 15159 | 0 | 100 |
| 3 | 80 | 5126 | 240 | 15378 | 0 | 100 |
| **Total** | 235 | 14006 | 705 | **42018** | 0 | 100 |

# Results: Larger-scale disinfestation Test at 3.1± 0.5º C

**Larger scale disinfestation test: Mortality of mature larvae (3rd instar) of *C. capitata*, inside Satsuma mandarin fruits at 3.1 º C on average**

| Replication | Control Fruits | | Treated Fruits | | | |
|---|---|---|---|---|---|---|
| | Nº Fruits | Living Insects | Nº Fruits | No. Estimated total treated insects | Total Nº of Survivors | Mortality % |
| 1 | 80 | 4501 | 240 | 13503 | 0 | 100 |
| 2 | 80 | 5145 | 240 | 15435 | 0 | 100 |
| 3 | 80 | 5078 | 240 | 15234 | 0 | 100 |
| **Total** | 240 | 14724 | 720 | **44172** | 0 | 100 |

# Conclusions: Larger-Scale Test

- According to the results of this work plan has been proven that cold strage quarantine treatment at an average of 2.1± 0.5º C for a period of 18-days and 3.1± 0.5º C for a period of 22-days in Satsuma mandarin, is efficient to completely mortality mature larvae (3rd instar) of the *C. capitata.*

- The estimated number of insects treated was 42,018 for 2.1± 0.5º C and 44,172 for 3.1± 0.5º C, respectively, above the estimated 30,000 individuals with zero survivors of the work plan.

# Results: Quality Control Test at 2.1± 0.5º C

**Quality Control Test: Evaluation parameters appearance, flavor, aroma, physiological and pathological changes in Satsuma mandarin under cold storage at 2.1° C, after 40 days.**

| Parameters | Time after treatment (days) | |
|---|---|---|
| | 0 Days | 40 Days |
| | Treated Fruits | Treated Fruits |
| Appearance | Good | Good |
| Aroma | Nice | Nice |
| Flavor (affected/total) (a) | 2 | 2 |
| Physiological changes (affected/total) (a) | 0 | 2 |
| Pathological changes (affected/total) (a) | 0 | 0 |

**Quality Control Test: Evaluation of qualitative characteristics in Satsuma mandarin under cold storage at 2.1° C, after 40 days.**

| Qualitative characteristics | Time after treatment (days) | |
|---|---|---|
| | 0 Days | 40 Days |
| | Treated Fruits | Treated Fruits |
| Weight loss (%) (b) | 0.0 | 5.8 |
| Brix Degrees | 10.17 | 9.94 |
| % Solubles Solids (b) | 10.5 | 10.5 |
| Titratable Acidity (b) | 0.88 | 0.93 |
| Maturity Index (b) | 11.98 | 11.4 |

(a): N° of fruits affected

(b): Media of 20 fruits

# Results: Quality Control Test at 3.1± 0.5º C

**Quality Contro Test: Evaluation parameters appearance, flavor, aroma, physiological and pathological changes in Satsuma mandarin under cold storage at 3.1 ± 0.5° C, during 35 davs.**

| Parameters | Time after treatment (days) | |
|---|---|---|
| | 0 Days | 35 Days |
| | Treated Fruits | Treated Fruits |
| Appearance | Good | Good |
| Aroma | Nice | Nice |
| Flavor (affected/total) (a) | 0 | 1 |
| Physiological changes (affected/total) (a) | 0 | 1 |
| Pathological changes (affected/total) (a) | 0 | 0 |

**Quality Control Test: EValuation of qualitative characteristics in Satsuma mandarin under cold storage at 3.1 ± 0.5° C, during 35 days.**

| Qualitative characteristics | Time after treatment (days) | |
|---|---|---|
| | 0 Days | 35 Days |
| | Treated Fruits | Treated Fruits |
| Weight loss (%) (b) | 0.0 | 3.9 |
| Brix Degrees | 10.35 | 10.02 |
| % Solubles Solids (b) | 10.1 | 11.6 |
| Titratable Acidity (b) | 0.94 | 1.00 |
| Maturity Index (b) | 10.7 | 11.5 |

(a): N° of fruits affected
(b): Media of 20 fruits

# Conclusions: Quality Control Test

- Cold treatment storage for a period of 40 and 35 days at an average temperature of 2.1º C and 3.1º C respectively at the core of the fruit, did not affect the quality of Satsuma mandarin, in regards to the proposed parameters, and the qualitative characteristics evaluated. This findings have confirmed the validity of this quarantine treatment for the export fruits acording to the requirements of international marketing

SENASA

equenta@senasa.gob.pe