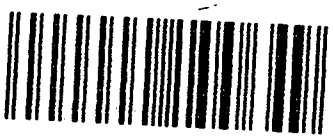
BARD

FINAL REPORT
PROJECT NO. I-749-84

Studies of Curing, Water-Saturated Atmosphere and Individual Seal-Packaging in Reducing Decay and Extending Life of Harvested Fruits

S. Ben-Yshoshua, J. W. Eckert

1989



30762 מס' ON

20-2-1989

Standard BARD Cover Page for Scientific Reports

Date: _____

BARD
P.O. BOX 6
Bet Dagan, ISRAEL

BARD project No. I-749-84

Title

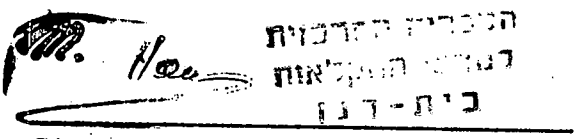
Studies of curing, water-saturated atmosphere and individual seal-packaging in reducing decay and extending life of harvested fruits.

Investigators' Names (Principal listed first)	Investigators' Institutions
S. Ben-Yehoshua	Department of Fruit and Vegetable Storage, ARO, The Volcani Center, Bet Dagan 50250, Israel;
J.W. Eckert	University of California, Riverside, California 92521-0122 U.S.A.

Project's starting date: _____

Type of Report: 1st Annual _____ 2nd Annual _____ Final x

S. Ben-Yehoshua
Signature
Principal Investigator



Signature
Institution's Authorizing Official

Page

B. Table of contents.

Page

C. ABSTRACT

Effects of water-saturated atmosphere, as achieved by the Filacell method, were compared with individual seal-packaging in maximizing the fruit's life, and reducing its physiological disorder and decay. Sealed lemons kept longer, stayed firmer and lost less weight than fruit in Filacell. The latter suffered from soilage after prolonged storage, the cartons in the Filacell collapsed, and the Bruce boxes were darkened by microbial contamination.

Water-saturated atmosphere achieved by either method was more important than temperature in slowing softening and physiological deterioration of bell pepper and various citrus fruits, but water saturation enhances decay of tomato.

A combined treatment of relatively high temperatures and seal-packaging-curing, reduced decay of various citrus fruits.

Optimization of the relevant parameters involved in the curing treatment enabled successful export of residue-free pomelo and lemons to distant markets.

The mode of action of curing in reducing decay was related to the thermic inhibition, to enhancing lignification and to the maintenance of higher levels of antifungal materials in the flavedo of fruit. This work demonstrated the presence of antifungal materials in citrus fruit and their involvement in the resistance against pathogens together with lignification, a fact which was previously refuted in the literature. The older the fruit is, either on or off the tree, the lower is both its resistance and its level of antifungal activity.

Adopting new techniques, the lignin-like materials synthesized in wounded citrus peel were identified as lignins. Their synthesis correlated in time with the increase in resistance of the wounded tissue to pathogens. Antifungal activity and lignification were most intense in water-saturated atmosphere which may explain the greater resistance of sealed fruit to pathogens.

D. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

a. Investigation of the effect of WSA and seal-packaging at various temperatures on specific physiological disorders and diseases of several fruits and vegetables, with emphasis on both the overall degree of decay and the specific effects of each of the pathogens involved.

b. Elucidation of the mode of action of curing in WSA to enhance healing and reduce decay, specifically at relatively high temperatures.

c. Comparison between the effects of WSA and individual seal-packaging in plastic film, on several crops including lemons and bell peppers.

d. Application of WSA as a preventative treatment to reduce physiological damage such as red blotch of lemons, and to enhance healing of injured fruit.

Work has been carried out on each of the four objectives. Since the research on curing gave the greatest success and seemed to be a "breakthrough" in the highly desirable option of eliciting the natural resistance of citrus fruit, all efforts were directed toward that goal. Thus, this work was emphasized and our results far exceeded our original objectives.

The research in Riverside centered on the correlation between the development of resistance in wounded citrus peel and lignification of the wound. The research in Israel was carried out by the tenured staff of this laboratory, Shimshon Ben-Yehoshua and Boris Shapiro: by Dr. Rami Moran, who joined us for an interim period between completion of

his doctoral dissertation and a postdoctoral in the U.S.A., Jonathan Sharoni, and Jong Jin Kim, a M.Sc. from Korea who received a CICAR scholarship and replaced Rami Moran. Four high school students performed a special research project on this BARD grant, one of which Noga Shem-Tov published her work in Hassadeh and in the Proceeding of the Citriculture Congress.

E. BODY OF THE REPORT

1. Comparative results in long-term storage of lemons seal-packaged and held in a Filacell.

One of the objectives of this project was to compare the effects of individual seal-packaging with those of the Filacell or Humifresh technique developed by Meredith. Both these methods, like the jacketed refrigeration of van-den-Berg and Lentz, were demonstrated to extend life of fruit for long periods by providing a water-saturated atmosphere. Lemons were selected as suitable to compare these techniques, because this fruit responds favorably to seal-packaging. Likewise, although lemons are available in Israel all year round, long-term storage is practiced because high prices are fetched during the summer, while most of the production is during December - January. Conventionally, lemons are stored at the relatively high temperature of 13°C, and lose their freshness and firmness after 2 months' storage; below 13°C the lemons suffer chilling injuries, and become unmarketable.

The Filacell rooms were designed in Israel by Eng. Asa Aharoni in accordance with Meredith's plans. These rooms

were provided with saturated humidity at a wide range of temperatures without water droplets being formed on the fruit. This was achieved by lessening the humidity and temperature gradient between the fruit and the ambient air. These two treatments were compared for 2 years. During the second year 3 tons of lemons were stored, half of them in a Filacell and the other half individually sealed, at 13°C. Additionally there were 5 cartons in 17° and in 13°C at 85-90% RH. This last treatment at both temperatures was kept only for 4 months, because the fruit became soft, shriveled and lost its commercial value. The Filacell or sealed fruit at both 13 and 17°C remained fresh and firm for an entire year. After 9 months storage at 13°C and 85% RH, the loss of weight of sealed fruit was 3% while that of nonsealed was 33%. The fruit in the Filacell lost much less weight than nonsealed but significantly more than sealed fruit. Thus after 1 month, sealed fruit lost 7.5% of the nonsealed and the Filacell lost 11.1% of the nonsealed. Furthermore, the firmness of the sealed fruit at both 13° and 17°C was higher than in the Filacell at 13°C. About 20% of the sealed fruits were resilient and 80% remained firm, whereas in the Filacell, 20% were soft, 60% were resilient and 20% were firm.

The major difference between the sealed and Filacelled fruit was in the decay and the resultant soilage. The percent decay after 9 months' storage was 16% for the Filacell and 14% and 17% for the sealed fruit at 13° and 17°C, respectively. However, the rotten sealed fruit could be culled and the rest remained marketable whereas the fruit in the Filacell was soiled and could not be sold without a difficult and tedious repackaging.

Another great difficulty with the Filacell was that the cartons absorbed the humidity and collapsed within several weeks. Even the Bruce boxes turned black due to microorganism contamination.

Thus, it appears that humidity and not temperature is the critical condition for the life and firmness of lemons. Probably, the parameter involved for the fruit is its water potential which remains in the sealed fruit at its original level of about -14 bars, whereas the nonsealed fruit declines to -21 bars already in the second month. Probably, the maintenance of a turgid condition, or the higher water potential inhibits the enzymes involved in the softening process, as shown for polygalacturonase by us in our previous BARD report. However, it should be noted that some other fruits, such as tomato, do not respond to the water potential as do lemons or peppers. They soften even under turgid conditions, without water stress - only at a slightly slower rate than under water stress. Furthermore, tomato also does not respond well to the water saturation as far as decay is concerned: that is, the tomato rots faster under saturated conditions than at lower humidities of 80% RH.

It appears that for some fruits, such as lemons and bell pepper, seal-packaging does provide the advantages of the water-saturated atmosphere and mitigates the disadvantages incurred by this high humidity, such as the increased decay brought about by water saturation and by the condensed water droplets formed on fruit or by the soilage spread by one rotten fruit over the rest, or by the collapse of the cartons and the blackening of the Bruce boxes in the Filacell. Of course, these advantages of seal-packaging should be considered along with the higher cost of this technique.

2. Comparing seal-packaging with the intermittent warming method for prolonged storage of lemons

In collaboration with Dr. E. Cohen, three treatments were checked to find the best conditions for extending lemon storage: non-sealed fruit at 14°C; HDPE-sealed fruit at 14°C; and non-sealed fruit held at temperatures alternating between 14 and 2°C. The aim of the last treatment, that was developed by E. Cohen, was to reduce the chilling injuries expected at 2°C by alternating the low temperature with 14°C. and to reduce the decay expected at 14°C by lowering the temperature to 2°C. The best treatment was the seal-packaging at 14°C, by which fruits could be kept for 10 months at 14°C, followed by 2 months of shelf-life. The non-sealed fruits that were subjected to alternating temperatures were firmer than nonsealed fruit kept at 14°C continuously but suffered from drying and softening as compared with sealed fruit. In fact, while sealed fruit lost over 6 months approximately 2% of its weight, the fruits subjected to the alternating temperatures lost 21% of their original weight and most of them were soft. It should be noted, though, that the intermittent warming developed by E. Cohen has already been practiced commercially for several years with great success in the control of chilling injury. However, these fruits could not be kept for as long as sealed fruit because of their loss of weight and softening; the packing house could operate this method but lacks the equipment to apply the sealing.

3. Physiological and chemical behavior of high density polyethylene-sealed lemons in long-term storage and shelf life (7, 20)

This work was done also in collaboration with E. Cohen and his laboratory (7, 20). We studied the effect of HDPE sealing on the physiological and chemical behavior of lemons from three different groves during storage, and compared it with that of nonsealed lemons. Color development in fruit picked at the mature green stage was slower during the first 2 months of storage in HDPE-sealed fruit than in nonsealed. Weight loss at the end of 6 months' storage plus 1 week of shelf-life was 7- to 10-fold less in sealed fruit. Sealed fruit had a lower water potential and less deformation than nonsealed fruit. The sealed fruit had a thicker peel, - both flavedo and albedo - and its segments contained slightly less juice and acid than nonsealed fruit. Electrolyte conductivity, respiration, ethylene production, and ethanol and acetaldehyde contents were lower in the juice of sealed fruit than of nonsealed. These results emphasized that maturation and senescence in HDPE-sealed lemons were slower and, as a consequence, after 6 months' storage the fruits were still of marketable quality.

Seal packaging even at 17°C gave better results than nonsealed fruit at 13°C, as expressed by firmness, weight loss and appearance.

4. The relationships among water-saturated atmosphere, seal-packaging and various blemishes and physiological disorders of citrus fruit

In our first BARD report we showed that seal-packaging reduced markedly the occurrence of the red blotch blemish of lemons and the stem-end rind breakdown of oranges. Our results were confirmed by many investigators all over the world. We have continued to search for better control of these blemishes during this subsequent BARD project. As will be shown in Section 10, both water-saturated atmosphere and high temperature involved in the curing treatment contribute to the reduction of various blemishes of citrus fruit. Independent of this project, Cohen et al. followed the curing treatment in our department and reported that raising the temperature of the degreening room of lemons from 25° to 30°C, controlled the red blotch. We also have followed the curing treatment of lemons and feel that raising the relative humidity that took place in addition to the raise of temperature in the degreening room contributes greatly to the control of the red blotch. Furthermore, high percentage of red blotch occurred at 30° and 25°C in nonsealed fruit in our experiments but very few in sealed fruit at both temperatures. The major difference between the sealed and nonsealed is that sealed fruit has water-saturated atmosphere. The nature and control of this disorder are not yet completely understood, although its incidence has been largely controlled in recent years.

Seal-packaging inhibited also the chilling injury of both grapefruit and lemons. It appears that sealed citrus fruit could withstand lower storage temperatures than nonsealed fruit does.

5. Effects of shrink seal-packaging with HDPE and Cryovac D-950 films on keeping quality of various citrus fruits(5)*

The effect of Cryovac D-950 film was investigated on the following citrus fruits: 'Shamouti' and 'Valencia' oranges, 'Marsh' grapefruit, 'Topaz' (Ortanique) and Mineola mandarin and 'Villafranca' lemons and Persian limes (5).

In similarity with high density polyethylene (HDPE) film, shrink seal-packaging with Cryovac D-950 and D-955 films delayed markedly the physiological deterioration of all fruits tested, as expressed in almost complete elimination of weight loss, shrinkage and deformation. The percent decay was also similar to that exerted by HDPE film. D-950 film, as well as new film, D-955, 0.015 mm thick, was slightly but significantly less permeable than 0.010-mm-thick HDPE. Fruit sealed with Cryovac D-950 had in general slightly more CO_2, C_2H_4, ethanol and acetaldehyde and less O_2, than non-sealed fruit or fruit sealed with HDPE; the latter had about the same levels of these materials as nonsealed fruit. The difference in permeability may be due to the greater thickness of the D-950 film as compared with the HDPE.

Unlike the slight effects on weight loss, the effects of waxing with Broshar on the composition of the

---

* This work was carried out in collaboration with the Agrotechnical Division of the Citrus Marketing Board and the Agricultural Extension Service of Israel.

intercellular atmosphere were greater than those of shrink seal-packaging with all films tested: HDPE, and Cryovac D-950 and D-955.

The important conclusion from these data is that waxing, and not seal-packaging, is the major factor that alters the composition of the micro-atmosphere, and consequently leads to risk of production of off-flavors due to the increase in CO_2, reduction of O_2 and marked increase in ethanol, acetaldehyde and many other volatiles. This is in fact the reason for the occurrence of off-flavor after too thick waxing or after maintaining waxed fruit at too high temperatures.

Both waxing as well as seal-packaging with D-950 or HDPE increased additively the percent of fruit with internal black rot caused by Alternaria citri and Fusarium sp. In the 1984/85 season the percent of these rots was high enough to prohibit the storage of Valencia oranges and Topaz mandarins beyond 6 weeks, at which time these rots appeared. It should be noted that the enhancement of these rots is very harmful commercially, since the rotten fruit cannot be culled out - as the decay develops first internally.

The findings confirm previous studies that indicate that seal-packaging could substitute for waxing; in fact, as far as D-950 is concerned, the two practices may be mutually exclusive.

The life of Mineola mandarin was doubled by shrink seal-packaging with HDPE or Cryovac D-950 or D-955 films. The extension of life was synergistic with the effect of an orchard spray with gibberellins that delayed the deterioration and softening of this fruit on the tree.

Seal-packaging of Persian limes delayed markedly their deterioration and the development of an undesirable yellow

color. This effect, too, was enhanced by a dip in gibberellin solution.

6. Cryovac sealed grapefruit: A taste and acceptability test

Marketing studies of sealed fruit were deemed essential for the judicious evaluation of this new technique. Consequently, we were happy to participate in such a project that was carried out by Prof. Arieh Goldman of the Hebrew University of Jerusalem with the support of the Israel Citrus Marketing Board.

The study was focused on testing the effect of the Cryovac seal on the taste evaluations, and on evaluating the acceptability levels of Cryovac-sealed grapefruit. Four grapefruit were defined for the test: (I) a non-sealed grapefruit of the type exported, served as the reference; (II) a Cryovac-sealed grapefruit that was sealed after it went through the regular packing house treatment; (III) a Cryovac-sealed grapefruit that went through only partial packing house treatment, free of waxing, and (IV) a non-sealed grapefruit that went through the same partial treatment as (III).

A sample of 135 respondents drawn from tourists visiting Israel (100 were from European countries) participated in the study. Each tester tested the above four types of grapefruit.

The subjects evaluated each of the grapefruit both in the context of a paired comparison and a direct order design. Each respondent also stated his/her intensity of preferences using a "dollar-metric" method; evaluated the profile of each grapefruit on a set of attributes; and

stated the profile of the "ideal" grapefruit on these attributes.

The results showed that (I) the sealed grapefruit achieved a high level of acceptability; (II) sealing of the fruit had a positive psychological effect on taste perceptions; and (III) among the two sealed grapefruits, the one that was sealed after passing only partial treatment in the packing house was judged to be the most preferred in taste.

7. Extending the life of bell pepper by a combined application of individual seal-packaging and various fungicides

This work was carried out with Naomi Temkin-Gorodeiski, ARO, and G. Gur of Agrexco.

Seal-packaging with two films of Cryovac (D-950 and D-955) gave results similar to HDPE in extending the physiological life of bell pepper. However, the transparent and glossy Cryovac films are much more consumer-attractive than HDPE.

Fumigation with nitrogen trichloride (Decco) reduced by 50% the decay caused by all three major pathogens of pepper: Alternaria alternata, Botrytis cinerea and the bacterium Erwinia carotovora. Similarly, imazalil was effective against A. alternata and, to a lesser extent, against B. cinerea, and slightly, if at all, effective against the bacterium. Imazalil and prochloraz were more effective than Decco and Panoctine (guazatine), and against Alternaria and Botrytis.

8. Shredded bell pepper - A new export product

The new "salad bar" emerging in many restaurants in the developed countries, requires new products of shredded vegetables. During the 1984/85 season several methods to develop shredded bell pepper (capsicum) were examined. The objective of this project was to increase the profit for the grower by marketing part of the produce that was culled because of size or color, even though its quality was suitable.

The shredded bell pepper was prepared in two forms, 1x5 cm strips or $1cm^3$ cubes, and then sealed in a vacuum package. The product kept long enough to allow air export to any part of the world. The cubed pepper kept less time than the strips, probably because of the greater area of the pepper that was injured. Cooling the shredded pepper with ice water immediately after preparation improved its keeping quality. Shredded pepper made of red fruit kept less well than that made of green fruit, although the flavor and appearance of the red fruit were better.

Disinfection of this product with 200-300 ppm active chlorine reduced markedly the bacterial contamination and extended also its life without leaving any residues of chlorine. The bacterial count was less than $1x10^5$ and the coliform count less than 50 per gram. The product was found to be of excellent quality in organoleptic tests that were carried out by us, by brokers abroad, and by the Central Food Laboratory of the Health Ministry.

Shredded onion was also prepared and found to keep well for air export, but the package emitted prohibitively onion odors.

These data were transferred to the Jordan Valley Development Authority to assist them in the establishment of a new commercial plant for the preparation of shredded vegetables. Future research should improve the keeping quality of bell-pepper and lead to the discovery of additional vegetables that could be utilized to extend the season of operation of this plant.

Two articles were published during the project (8, 9) based in large part on data collected in the first BARD project. However, these data were verified and summarized during the current project.

9. Rotronic probe gives accurate measurements of high humidity in a sealed enclosure with fruit

Despite the attempts of many researchers, accurate readings could not be made in the past of the exact humidity inside the sealed enclosure. Usually the probe would accumulate condensed water droplets soon after its insertion into the sealed enclosure with a fruit. These water droplets would affect the humidity reading. Only recently were we able to overcome this difficulty with the new electronic gadget from Rotronic, Switzerland. This gadget gave us an accurate reading of the humidity inside the sealed enclosure and could detect real differences in the RH inside different plastic films. We hope that the probe will be of great use in the further development of this field of accurate measurement of a high range of humidities close to water saturation.

10. Individual seal-packaging enables the use of curing at high temperature to reduce decay and heal injury of citrus fruits (3, 6, 10, 11)

The marked extension of life of citrus fruit by individual seal-packaging shown in a previous BARD report has attracted both basic and commercial interest. Delayed physiological deterioration by seal-packaging was related to the alleviation of water stress of the harvested fruit. However, a risk from this technique is the possibility that decay might be enhanced by the water-saturated atmosphere of the sealed fruit. Although most reports do not describe any marked effect on decay, several noted that the percent decay of 'Shamouti' and 'Valencia' oranges was slightly increased by seal-packaging. This increase could be a function of time, since the storage life of sealed fruit is longer than that of nonsealed fruit. Sealing of citrus fruit increased stem-end rot in general and Alternaria rot in particular However, the pathogen causing this type of decay usually requires 8 weeks for incubation, longer than fruits are usually kept in sealed packages. The major pathogen causing decay of citrus is still the green mold, Penicillium digitatum Sacc.

Curing has been used commercially for several decades for potato tubers, sweet potato roots, and several kinds of flower bulbs. The process involves keeping the produce at relatively high temperatures (15 to 40°C) and > 95% RH.

Hopkins and Loucks in 1948 showed that Penicillium decay of Florida oranges could be reduced by keeping fruit for several days after harvest at 30°C and 90-100% RH. However, other types of decay rose. Brown and co-workers (11) found these conditions to elicit the biosynthesis of