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ENHANCEMENT OF FRUIT AROMA AND QUALITY BY POSTHARVEST PRETREATMENTS WITH ANAEROBIOSIS OR ANAEROBIC METABOLITES

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Executive Summary:

During the second year, we continued to develop in both countries new biological-organic compounds that can be applied as postharvest solutions to improve fruit quality. Because the apple is eaten with its peel it is a must that all compounds used will be organic and edible. In Kazakhstan we treated with various organic compounds two main apple cultivars Aport and Zarya Alatau. They had the best results in apples treated with bee glue, that exhibited a very low percent of losses after 7 months at 0C, was accepted. The application to apples of film-cover coating which includes a bio-active material with different action spectrums allowed to reduce apple fruit losses due to the decay development and prevented functional disorders. In Israel we used two new organic coating on apples cvs. Starking and Golden Delicious. One of them is a colloidal solution based on beeswax and the other is based on carnauba wax (a wax extracted from a palm tree in Brazil). Both coatings maintain the apples appearance in a better shape for a long time. Moreover, the coated apples kept better taste, because of a lower production of the anaerobic metabolites – acetaldehyde and ethanol.

Another subject that we studied in Israel was improving fruit quality by application of a novel gas, 1-methylcyclopropene (MCP) which is an ethylene inhibitor who acts by binding to the cellular ethylene receptors. We tried it on grape fruit which is non climacteric fruit and also on bunches of cherry tomato which are climacteric fruit. On grapes MCP did exhibit good effect in preserving its posthravest life, contrary it caused more damage and caused acceleration of anaerobic metabolites. However, on tomato it was effective in reducing fruit shattering and ripening.

Section I

A. Research Objectives

The major objective is to develop and apply post-harvest technologies to extend the storage and the shelf life of fruits and to maintain their quality. The specific objectives include:

- Develop and apply fruit coating that will prevent fruit senescence, maintain fruit aroma and taste, and enhance resistance against injury and fungus development.
- To explore the potential of bioactive substances of vegetative and synthetic origin that improve the quality of the fruits.
- To study the influence of MCP treatment on tomato and grape clusters
- To study the role of low O_2 pretreatment for inhibition of scald appearance in apples.

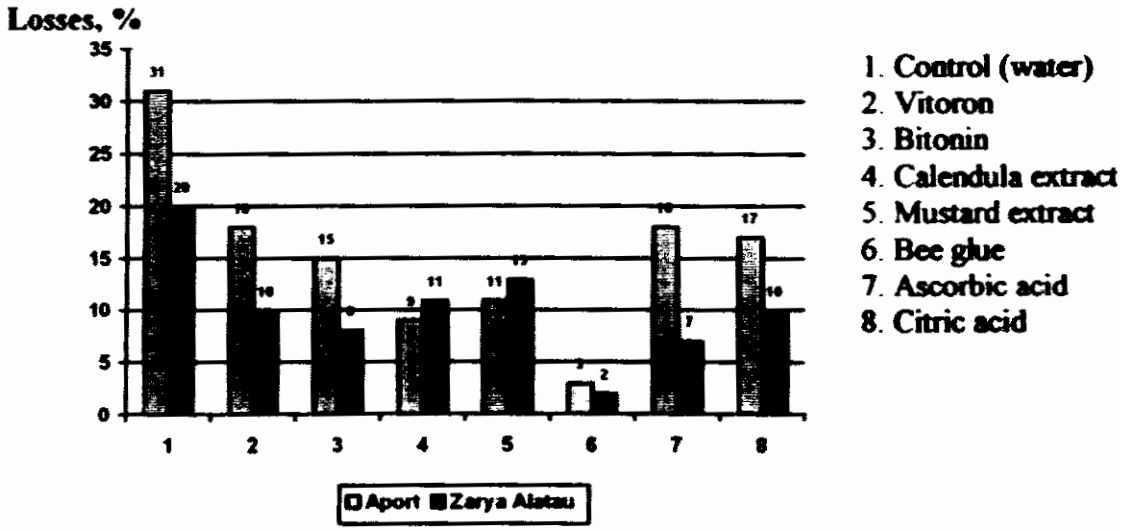
B. Research accomplishments

Organic treatments

The investigations in Kazakhstan were conducted on apple fruits of the local varieties Aport and Zarya Alatau. The fruits of 'Aport' are of high trade quality, but they are subjected to fungal diseases during storage. The fruits of 'Zarya Alatau' are affected by functional disorders – apple bitter pit and cuticular browning.

The degree of fruit ripening was determined according to colour, size, iodine starch test and organoleptic values. The apple fruits were treated with various organic solutions that are created from natural sources rich with antioxidant activity (Fig. 1). The best result was obtained in fruit treated with the compound no. 6. which is the bee glue. The bee glue is an extract from the beehive that probably posses fungicidic activity and was very effective on both cultivars (Fig. 1). The fruit treated with bee glue showed a very low percent of losses after 7 months at 0C.

Fig.1. The effect of various bio preparations on percent of apple fruit losses in cvs. Aport and Zarya Alatau after 7 months of storage at 0C.



The losses from diseases in the bee glue-treated were 6-10 times lower than in the control. Taste value, conducted after removal from 7 months cold storage confirmed the fact that the apple fruits treated by bio preparations were better than the non-treated fruits according to flavour quality (Table 1).

Table 1. Effect of various bio preparations on taste indices of two apple cultivars Aport and Zarya Alatau, after 7 months of storage at 0C.
Taste index (1= worst taste; 10 = best taste).

Experimental variants	Taste Index (1-10)	
	Aport	Zarya Alatau
1. Control (water)	5,5	5,8
2. Vitoron	6,5	7,0
3. Bitonin	7,0	7,5
4. Colendula extract	7,1	6,8
5. Mustard extract	6,7	6,3
6. Bee glue	8,1	8,5
7. Ascorbic acid	6,8	7,2
8. Citric acid	6,8	7,1

In Israel we used two organic coatings for post-harvest application in fruits. One coating is a colloidal solution based on beeswax (Developed by the Israeli company 'Biocoat') and the other is based on carnauba wax (Developed by the American company 'Decco'). Both organic waxes are in the product list of the Organic Materials Review Institute (OMRI) based in Oregon, USA (www.omri.org). The main distinguishing feature between the two wax coatings is that beeswax coated fruit had a lusterless look, while the carnauba wax coated fruit exhibited a shiny appearance. Application of the organic waxes to two cultivars of apples Golden Delicious and Starking improved their storability in cold and in shelf life. After 1 month at 20C, in both cultivars the coated fruits had higher firmness and lower anaerobic volatiles production of acetaldehyde (AA) and ethanol (Fig. 2). In Starking the coated- apples had higher TSS values, but in Golden Delicious coating the fruit inhibited sugar accumulation and the coated fruits had lower TSS than the uncoated. In both cultivars after 1 month at 20C the levels of the anaerobic metabolites, AA and ethanol, was much lower than the levels that was found in the uncoated fruit (Table 2). The lower levels of anaerobic metabolites indicate that the coating did not caused blockage in the gas exchange. The taste test revealed that in both cultivars higher percent of the panelists preffered the coated fruit, probably because of the lower anaerobic metabolites.

Fig. 2. Effect of various waxing in apple cvs. Starking and Golden Delicious on the firmness and total soluble solids (TSS) after 1 month storage at 20C.

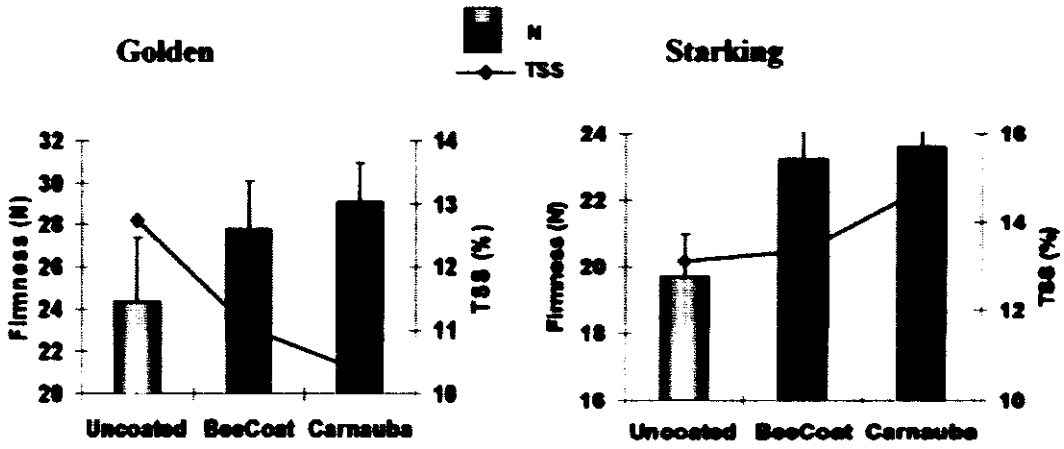


Table 2. Effect of two organic coatings (Beecoat and Carnauba) on the accumulation of acetaldehyde and ethanol and percent taste preference of apples cvs. Golden Delicious and Starking after 1 month at 20°C.

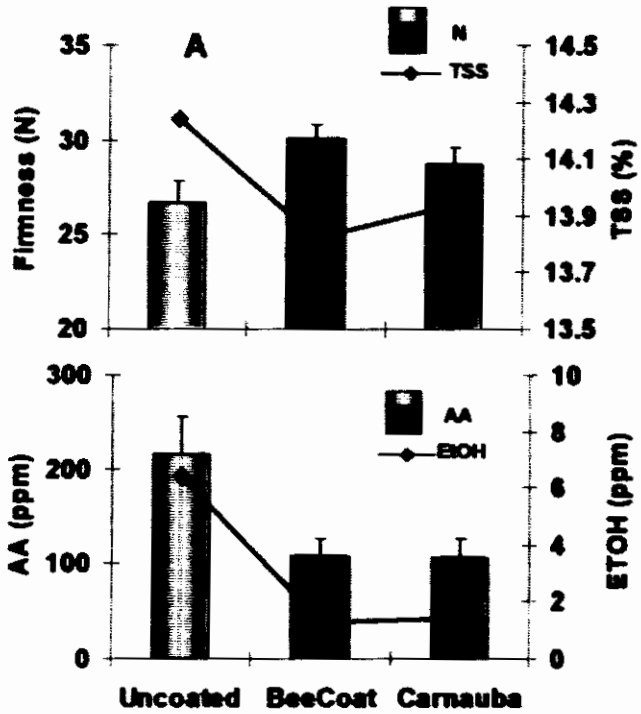
	Golden Delicious			Starking		
Trt.	AA (ppm)	Ethanol (ppm)	Percent preference	AA (ppm)	Ethanol ppm)	Percent preference
Uncoated	81.6 a	45.7 a	19.0	36.9 a	77.3 a	23.0
BeeCoat	38.8 b	36.9 b	48.0	32.5 a	38.1 b	41.0
Carnauba	37.7 b	29.6 c	33.0	23.2 b	36.3 b	36.0

The apple fruits were kept for long storage in regular air. Apple cv. Starking was kept for 5 months at 0°C and then transferred for 8 days at 20°C. The coated fruit suffered from less weight loss and decay during cold storage and shelf life (Table 3).

Table 3. Effect of two organic coatings (Beecoat and Carnauba) on the percent of weight loss and the decay of Starking apple after 5 months of storage at 0°C following 1 week at 20°C.

	Weight Loss (%)		Decay (%)	
Trt.	5mo at 0°C	+1wk at 20°C	5mo at 0°C	+1wk at 20°C
Uncoated	2.98 ± 0.24	3.47 ± 0.27	15.38 ± 3.42	16.67 ± 5.56
BeeCoat	2.72 ± 0.14	3.05 ± 0.12	8.33 ± 1.86	11.26 ± 1.37
Carnauba	2.09 ± 0.07	2.40 ± 0.07	5.13 ± 2.28	7.14 ± 2.38

The coated fruit maintained better firmness after the long storage and shelf life. The uncoated Starking had higher TSS levels but also much higher levels of AA and ethanol which worsen their taste (Fig. 3). The Carnauba coated Starking had the best taste, with 73% of the panelists preferring it (Fig. 3). The Starking that was coated with Beecoat got the lowest preference score probably because the coating inhibited the normal aroma volatiles that contribute to the aroma.



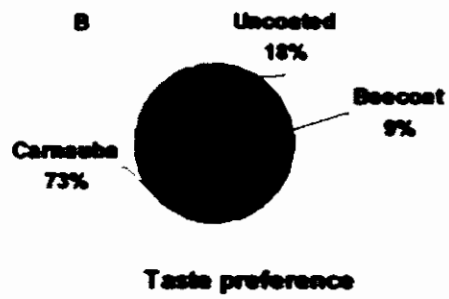


Fig. 3. Effect of various waxs in apple cv. Starking on: the firmness, total soluble solids (TSS), the levels of endogenous gases: acetaldehyde (AA) and ethanol (ETOH) (A) and the taste preference (%) after 5 months at 0°C + 8d 20°C (B).

Golden Delicous apples were kept for 7.5 months at 0°C in regular air and then transferred for 1 wk to 20°C. Once more, the Carnauba coated fruit exhibited the best appearance. It did not shrink during the long storage and also kept the greenish color without any rot development (Table 4). However, the Carnauba coated Golden exhibited the lower levels of TSS which influence the taste and the Beecoat coated Golden had the highest score of taste preference (Fig. 4). Also in Golden Delicious after long storage the levels of AA and ethanol were significantly lower than in the uncoated fruits (Fig. 4).

Table 4. Effect of two organic coatings (Beecoat and Carnauba) on the color index (1= green; 10=yellow), and the percentage of shrinking and decay in Golden Delicious apple after 7.5 months of storage at 0°C following 1 week at 20°C.

	Color (Index)	Shrinking (%)	Decay (%)
Control	7.3 ± 0.05	38.2 ± 5.13	17.2 ± 0.51
BeeCoat	6.8 ± 0.23	26.9 ± 3.85	7.7 ± 0.14
Carnauba	5.1 ± 0.23	26.8 ± 1.79	0.0

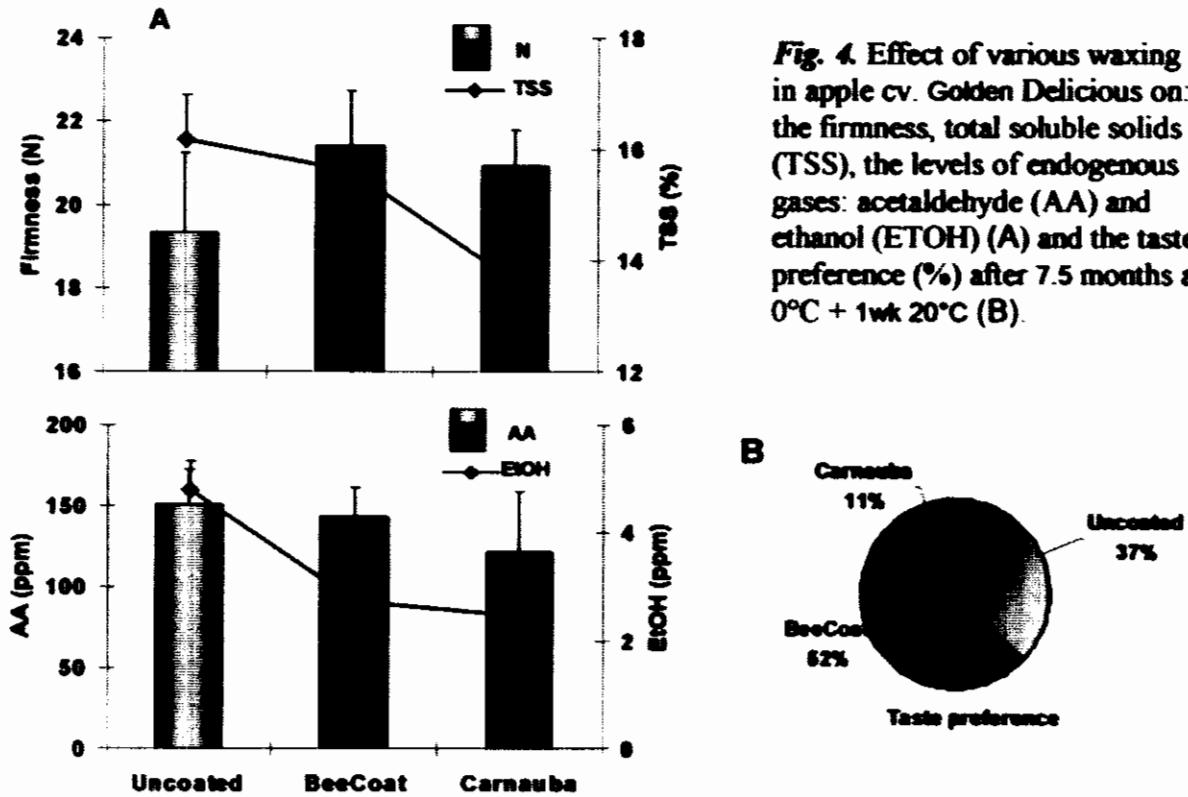


Fig. 4. Effect of various waxing in apple cv. Golden Delicious on: the firmness, total soluble solids (TSS), the levels of endogenous gases: acetaldehyde (AA) and ethanol (ETOH) (A) and the taste preference (%) after 7.5 months at 0°C + 1wk 20°C (B).

The interesting results is that storing Starking or Golden Delicous immediately at 20°C did not cause accumulation of great quantities of AA and the main volatile was ethanol, which is the end product in the anaerobic respiration. However, storing the fruits at 0°C for several months and removing them for 1 wk at 20°C caused a dramatic accumulation of AA, and very minor amounts of ethanol (Figs. 3 & 4). It is probable that after the long storage the apple lost its ability to convert AA to other metabolites such as ethanol or esters via acetyl CoA and therefore the amount of AA increased.

The levels of AA after one month at 20°C was only 35-70 ppm, while after cold storage and 1wk at 20°C, it reached around 200 ppm in both cultivars. On the other hand, the levels of ethanol after 1 month at 20°C was around 50-70 ppm, while after cold storage and 1 wk at 20°C it was only around 5 ppm,

After one month storage in both cultivars, the two different coated apples got similar percentage of preference in the taste panel. However, after the long storage in Starking fruit, the panelists preferred the Carnauba coated apples in a very significant way, while in Golden fruit they preferred the BeeCoat coated fruit (Figs. 3 & 4). The main distinguishing feature between the two wax coatings is that beeswax coated fruit had a lusterless look, while the carnauba wax coated fruit exhibited a shiny appearance. But both of them were effective in inhibiting senescence in apples after long periods of cold storage.

MCP treatments

A novel gas, 1-methylcyclopropene (MCP) was reported to inhibit ethylene action by binding to the cellular ethylene receptors, and to inhibit ethylene responses effectively in various fruits (Sisler et al. 1995; Sisler and Serek 1997). We have shown that MCP is an effective molecule when applied to climacteric fruit that

produce ethylene such as avocado and tomato (Pesis et al. 2002; Beno-Mualem et al. 2004).

In the literature there are few studies that show beneficial effect of MCP on non climacteric fruits such as pineapple (Selvarajah et al. 2001). We tried application of MCP to grape berries by using it in modified atmosphere (MA) packaging. We applied MCP (1ppm) to grapes cv. Superior packed in polyethylene (PE) or Xtend film (XF) and stored at 5°C which formed MA conditions, in order to see if it reduces fungal development or fermentation. Grape is a non climacteric fruit so the different accumulation of CO_2 in the packaging is due to the different permeability of the film. The XF film is less permeable so the levels of CO_2 reached around 2%, while in the PE film it reached only up to 4%. There was no difference in the CO_2 accumulation in the MCP treated fruit compared to the untreated (Fig. 5A).

The addition of MCP to the packed grapes induced the immediate production of ethylene in the MCP treated grape, which remained higher during 3 weeks of storage at 5°C especially in the less permeable XF film (Fig. 5B). The MCP stimulated the ethylene production which probably caused some injury to the grapes (Fig. 5C). Accumulation of anaerobic metabolites because of MCP treatment was previously shown in citrus which are also non climacteric fruit (Porat et al. 1998).

After 24 days at 5°C the bags were opened for quality check of the grape berries (Table 5). Grape berries that were packed in PE bags did not lose from their weight, however, they suffered from more decay development. On the other hand, fruits that were stored in XF film, lost some weight, but showed lower decay development (Table 5). The control grape berries that were not wrapped suffered from the highest weight loss and the rachies appeared much drier but with the lowest decay development (Table 5). The MCP-treated berries packed in PE or XF film suffered from more decay development than the berries packed in MA alone, probably because MCP caused more damage to non-climacteric fruits as was shown previously in citrus (Porat et al. 1998). Checking the taste of the MA treatments compared to the control, revealed that the best taste was found in berries from the XF packaging, they were more sweet and less sour and 50% of the panelists preferred this treatment (Table 5).

Table 5. Effect of film type and MCP on percent weight loss (WL), decay development, and taste indices (1-10) of sweetness and sourness, and percent preference of the taste panelist after 24 days at 5°C.

	WL %	Decay %	Sweetness Index	Sourness Index	Preference %
PE	0.00	5.86	6.57	3.62	25.00
MCP-PE	0.00	7.88			
XF	0.45	2.49	7.36	2.85	50.00
MCP-XF	0.39	6.60			
Control	4.02	2.31	7.00	3.45	25.00

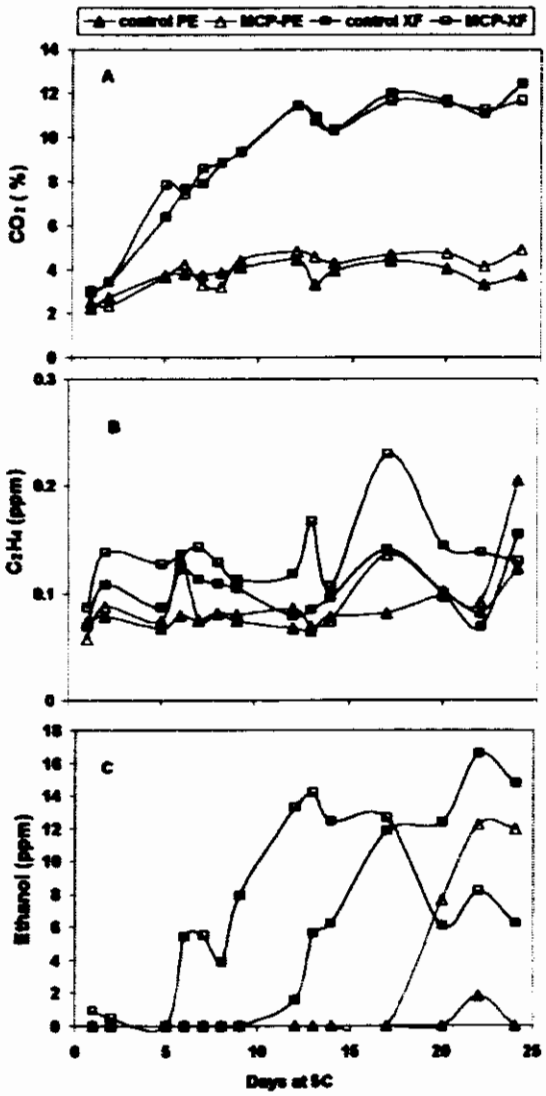


Fig. 5. **Effect of different bags wrapping + MCP treatment in grape cv. Superior on the levels of carbon dioxide (CO_2), ethylene (C_2H_4) and ethanol during cold storage at 5°C.**

The effect of MCP treatments on ripening and abscission of cherry tomatoes harvested as bunches is detailed in the manuscript by Beno-Mualem et al. (2004).

C. Scientific Impact of Collaboration:

During the second year of collaboration there was a lot of exchange of information and results. On Feb 2003, Ms. Tamara Nikitinko arrived as a trainee from Kazakhstan and stayed in the Israeli lab until Dec 2003. She studied a lot of new techniques such as using the gas chromatograph (GC) to measure volatiles. She now knows how to apply the various treatments, to check the results and analyze the data. Now, she is back in Kazakhstan teaching what she studied in Israel. In addition on Sep 2003, Dr. Uruopina arrived to visit the lab in Israel for 2 weeks. We discuss together the results and the plans for the future. On April 2004, Ms. Oxana Gadiva arrived as a second trainee from Kazakhstan to work in the Israeli lab for a period of one year.

D. Description of Project Impact

During the second year we continued as in the first year to work in both countries on apple coatings. The coatings were effective against dehydration and maintained better firmness. These coatings which are based on natural-organic compounds have also some antifungal effect and did not cause accumulation of

fermentative metabolites - AA and ethanol. Moreover, the coatings improved fruit aroma and taste after long cold storage.

During the second year we concentrated in Israel on working on inhibiting fruit senescence by application of the ethylene inhibitor MCP. It was an effective treatment to improve cherry tomato quality, to prevent fruit shattering from the bunches. In grapes, which is a non climacteric fruit, MCP was not effective and caused more damage. However, we found that storing grapes in XF film after ethanol treatment can be an alternative to the use of SO_2 in grape storage.

E. Strengthening of Developing Country Institutions:

Ms. Tamara Nikitinko that spent a period of time as a trainee in the lab in Israel went back to Kazakhstan where she is teaching the people in the lab all the new technologies she studied in Israel. However, the main instrument that is needed in Kazakhstan is a gas chromatograph (GC) so they can measure the volatiles that come out from the fruit. So far they did not purchase the GC, which is very critical for continuing the project.

F. Future work

In both countries we will continue to develop the optimal fruit coating for various fruit commodities. We will study the effect of these coating on maintaining fruit quality, aroma and taste. We will study the fungicidic effect of the various waxes and bioactive materials.

We are planning to continue the work with MCP on various fruits in order to delay their senescence. In grape clusters we will study the effect of SO_2 or ethanol in combination with modified atmosphere for improving grape quality.

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Section II

A. Managerial Issues

There was a big delay in the starting of the project and the arrival of the first trainee from Kazakhstan (because of the war in Iraq). This is the reason we asked for an extension of the project to another year (without addition of money). Now the project is extended until the end of August 2005 and we want to finish all our plans up to that date.

B. Budget

The main problem is transferring the money for the project to Kazakhstan. Although we sent the budget report on Jan 2004 and request for another payment it still did not arrive. Dr. Uryupina provided the explanation for all the expenses they made but so far, they did not get another payment for purchasing the GC. The group in Kazakhstan must purchase a GC for the project and they need the money for this reason. They already spent money from other source to send the second trainee Ms. Oxana Gadiyeva to Israel.

C. Special Concerns

The project is going very well and we already got significant results. However, both places, Israel and Kazakhstan, are not using the entire budget. In the last year of the project we intend to implement our research in semi-commercial experiments, and to purchase a GC for Kazakhstan, so we need to liquidize more of the available money.

D. Collaboration

Dr. Uryupina already visited Israel on Sep 2003. During her visit we spent time in the lab and went together to the agriculture exhibition (Tel Aviv) and packinghouse (North of Israel). Because Dr. Uryupina's English is not so good Mr. Oleg Feygenberg, from the lab of Dr. Pesis, translated to Russian all about the research we are conducting in Israel and clarified all the processes occurred in the packinghouse from harvesting, sorting, packing, storage and marketing. The team from Israel intends to go to Kazakhstan on Sep. 2004. To make the visit more effective Dr. Pesis already asked for the approval that Mr. Oleg Fygenberg, who is a M.Sc. Research Engineer, will join Dr. Pesis and Dr. Lichter. In addition, because of the poor English of the people in Kazakhstan we are sure that only if we will come with someone that speaks Russian it will be possible to explain all about our technology.

E. Request for American Embassy actions

a. The first request is to send the reimbursement payment to Kazakhstan so it will be possible for them to purchase the GC which is so needed to the continuity of the project. They sent a new request for Varian GC which is American made and which costs $ 28,000.
b. To approve the visit of M.Sc. Oleg Fygeneberg from Israel to Kazakhstan, so the visit of the Israeli team will be more effective.