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

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3. Executive Summary:

During the four years of the project we studied various postharvest technologies in order to increase storability and fruit quality. The principle directions were: 1. fruit coating with various organic waxes or organic antioxidants compounds; 2. application of low O_2 atmosphere prior to regular storage to apple in order to reduce scald damage. 3. application of 1-methylcyclopropene (1-MCP), the ethylene action inhibitor, in order to prevent chilling injury symptoms in various fruit. 4. replacement of SO_2 treatment in grape for decay control with ethanol treatments prior to and during storage.

The application to apples of film-cover coating which includes bio-active material with different action spectrums allowed to reduce apple fruit losses due to decay development and prevented physiological disorders. In Kazakhstan we treated with various organic compounds two main apple cultivars Aport and Zarya Alatau. The best results were obtained with apples treated by bee glue resulting in a very low percent of losses after 7 months at 0C. In Israel we used two new organic coating on apples cvs. Starking and Golden Delicious: one of them is a colloidal solution based on bees wax 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 retained better taste, due to lower production of the anaerobic metabolites – acetaldehyde and ethanol.

Sealing Granny Smith apples in a low-O_2 atmosphere for 7 days at 20°C (LO_2-7d), prior to cold storage reduced superficial scald development after 6 and 8 months at 0°C plus two weeks at 20°C. The LO_2-7d treatment reduced the O_2 levels that induce endogenous production of significant amounts of CO_2, acetaldehyde and ethanol, and thereby reduced ethylene production during cold storage, which, in turn reduced scald development. Treatments with ethanol vapor or low O_2 for 1 day at 20°C plus 6 days at 0°C (LO_2-1d) did not reduce scald effectively; 1-MCP treatment at 0.5 $\mu l\ l^{-1}$ was the most efficient in reducing the scald development. The reduction in scald development was associated also with lower peroxidase (POD) and polyphenol oxidase (PPO) activity in the peel of LO_2-7d and MCP pretreated fruits. The levels of α-farnesene and the conjugated trienols (CTs) were significantly lower in the 1-MCP-treated fruits than in those from the other treatments after 6 months of storage at 0°C, which was correlated to the minimized scald development. A simple treatment (LO_2-7d) at 20°C prior to regular cold storage could be suitable also for organic apples that cannot be treated with DPA or 1-MCP. One of the advantages of this method is its lower cost compared to low O_2 and CA storage for 6-8 months.

Dipping grape clusters in 25-35% ethanol solution disinfected the berries from pathogens and resulted in better quality of grape clusters cv. Taifi Rosoviy with less

berry shatter and delayed skin browning. Dipping grape bunches cv. Thompson seedless or cv. Superior in ethanol solution followed by storage in modified atmosphere using different films, kept the grape in excellent condition. It seems that part of the effectiveness of the ethanol is due to the formation of acetaldehyde (AA). Application of AA vapors was very effective in prevention development of fungi *in vitro.*

4. Research Objectives:

The overall aim of the project is to improve fruit quality and reduce decay development in grape, apple and tomato and to extend the duration of storage and shelf life duration.

A subsidiary objective is to study the metabolic basis for inhibition of ripening by anaerobic metabolites either naturally induced by anaerobic conditions following exposure to high CO_2 or low O_2 atmosphere, or by the application of ethanol. This objective was investigated by studying:

1. The interaction between anaerobic metabolites and ethylene formation on inhibition of scald development in apples.
2. The effect of treatments with anaerobic conditions on aroma volatiles production, color development, chemical composition of soluble carbohydrates and organic acids.
3. The effect of treatments with anaerobic conditions on induction of natural antioxidants and antioxidative enzymes.
4. The effect of ethanol treatments on decay development in grape.
5. The effect of fruit coating and wrapping on creation of modified atmosphere for extending the storability and shelf life of apple and melon.

5. Methods and Results:

5.1. Prevention of apple scald by low O_2 or 1-MCP pretreatment

The development of scald which is a result of chilling injury in apple is caused by the autooxidation products of α-farnesene, identified as conjugated trienes and 6-methyl-5-hepten-2-one, in the apple skin (Mir et al., 1999). It was shown earlier that the synthesis of α-farnesene was influenced by ethylene (Watkins et al., 1993). Recently, Zanella (2003) has shown in comparable research on apple that scald development was controlled by long-term storage under 0.7 % O_2, followed by storage under 1.0 % O_2, or by the application of 1-MCP at 1 ppm.

In order to reduce scald in apples we conducted experiments including pretreatments with low oxygen atmospheres and 1-MCP prior long storage at 0°C.

5.1.1. Fruits and treatments

Granny Smith apples were brought from a packinghouse in the north of Israel on the day of picking, and were sealed, a day later, in 30-L jars (10 kg/jar) with various gas atmospheres for 7 days. The treatments included: control (ambient air), ethanol vapour 2 %, low O_2 at 20°C for 1 day (LO_2-1d), low O_2 at 20°C for 7 days (LO_2-7d). Control, ethanol and LO_2-1d treatments were enclosed for 1 day at 20°C and remained closed for another 6 days at 1°C. Ethanol was applied by enclosing the fruits for 24 h at 20°C in 30-L containers with a beaker containing 80 % ethanol solution and a wick to aid evaporation; this generated approximately 2 % ethanol

concentration in the head space. The low-O_2 treatment was applied by flushing the containers with N_2 from a cylinder until the O_2 concentration fell to 3% and then closing the jars for 1 or 7 d at 20°C. After 24 h three of the low-O_2 jars were removed to 1°C for an additional 6 days (LO_2-1d) and three jars were kept at 20°C for a total of 7 d at 20°C (LO_2-7d). After 7 days all fruits were removed from the sealed jars and transferred to plastic punnets in cartons (Fig. 3) for continuous storage at 0°C in regular air for 6.5 months followed by 10 days of shelf life at 20°C.

In a second experiment, in the following season, Granny Smith apples were sealed in 250-L containers in the appropriate gas atmospheres for the various treatments: control; ethanol vapour, 2%; low O_2-1d; low O_2-7d; and 1-MCP, 0.5 µl l^{-1} (SmartFresh™). All treatments apart from low O_2-7d were applied for 24 h at 20°C, after which the fruits were transferred to plastic punnets in cartons for long cold storage at O°C in regular air atmosphere. The LO_2-7d-treated apples remained under low O_2 at 20°C for 7 d before the jars were opened and fruits transferred to cartons for continuous storage in regular air at 0°C.

In the first experiment fruit were sampled after 1 month and after 6.5 months of regular cold storage at 0°C plus 10 days of shelf life at 20°C. In the second experiment fruit were sampled after 6 and 8 months of regular cold storage at 0°C plus 10 days of shelf life at 20°C.

5.1.2. Gas concentrations during treatments

Table 1 summarizes the levels of CO_2, O_2, ethylene, AA and ethanol that accumulated in the headspace in the various treatments after 7 d at 20 and/or 1°C in the first experiment. In the low-O_2 treatments the levels of O_2 were very low, and it dropped to a minimal level of 0.38% after 7 d at 20°C (LO_2-7d) (Table 1); in this treatment there was more accumulation of AA and ethanol than in the (LO_2-1d) treatment, in which there was only 1 d at 20°C and 6 d at 1°C (Table 1). The LO_2-7d -treated fruits produced the highest amount of CO_2 (13.13%) during treatment (Table 1). In the ethanol-treated fruit the levels of AA and ethanol were very high, but the levels of O_2 were as in the control treatment, and there was some production of ethylene (Table 1).

Table 1. Effects of ethanol vapour (2%), LO_2-1d and LO_2-7d pretreatments on the gas levels measured in the headspace of 30-L jars after 7 days of treatment. Treatments 1-3 were enclosed in jars for 1 day at 20°C plus 6 days at 1°C; treatment no. 4 was enclosed for 7 days at 20°C.

Treatments	CO_2 (%)	O_2 (%)	AA (µl l^{-1})	EtOH (µl l^{-1})	C_2H_4 (µl l^{-1})
1. Control	5.91 b*	15.19 a	1.53 c	2.1 d	0.08 a
2. Ethanol	1.63 c	15.88 a	88.42 a	5522.5 a	0.08 a
3. LO_2 -1d	3.73 b	1.13 b	1.59 c	13.2 c	0 b
4. LO_2 -7d	13.13 a	0.38 c	9.43 b	135.1 b	0 b

*Values within each column followed by the same letter do not differ significantly according to Tukey's Test ($P < 0.05$, n = 3).

5.1.2. Volatiles accumulation after cold storage

After 1 month at 0°C the highest levels of endogenous AA and ethanol in apple disks from fruit of the first experiment were in the ethanol- and the (LO_2-7d)-pretreated fruits (Table 2). Following 10 days at 20°C, the levels of ethanol were very low in all treatments except in (LO_2-1d)-pretreated apples, in which the levels of ethanol increased after shelf life from 93.56 to 295.6 $\mu l\ l^{-1}$ (Table 2). On the other hand, in the (LO_2-7d)-pretreated fruit the fruits metabolized the high level of ethanol (786 $\mu l\ l^{-1}$) during shelf life and reduced it to a lower level than in the control fruit (36 $\mu l\ l^{-1}$) (Table 2).

Table 2. Effects of ethanol vapour (2%), LO_2-1d and LO_2-7d pretreatments on acetaldehyde (AA) and ethanol (EtOH) production in Granny Smith pulp disks after 1 month at 0°C and 10 d at 20°C.

Treatments	1 month at 0°C		10 days at 20°C	
	AA ($\mu l\ l^{-1}$)	EtOH ($\mu l\ l^{-1}$)	AA ($\mu l\ l^{-1}$)	EtOH ($\mu l\ l^{-1}$)
Control	17.87 b*	39.50 d	29.02 a	52.61 b
Ethanol	35.54 a	285.89 b	26.90 a	53.80 b
LO_2-1d	18.11 b	93.56 c	23.14 a	295.64 a
LO_2-7d	36.03 a	786.80 a	20.94 a	36.18 c

* as Tab 1

The level of ethylene present in disks of apples from the LO_2-7d treatment was significantly lower than in all other treatment after 6.5 months at 0°C (Table 3). This low ethylene concentration was correlated with the highest AA and ethanol levels in these fruits on the day of removal from cold storage (Table 3). The levels of AA increased five- to tenfold in all treatments during 6.5 months at 0°C, whereas those of ethanol decreased dramatically – to less than 10 $\mu l\ l^{-1}$ – in all treatments, compared with the levels found after 1 month at 0°C (Tables 3 and 2, respectively). In all treatments the level of ethylene increased but those of AA and ethanol decreased, during 10 d of shelf life at 20°C (Table 3).

Table 3. Effects of ethanol vapour (2%), LO_2-1d and LO_2-7d pretreatments on ethylene, acetaldehyde (AA) and ethanol (EtOH) production in Granny Smith pulp disks after 6.5 month at 0°C and 10 d at 20°C.

Treatments	6.5 months at 0°C			+ 10 days at 20°C		
	Ethylene ($\mu l\ l^{-1}$)	AA ($\mu l\ l^{-1}$)	EtOH ($\mu l\ l^{-1}$)	Ethylene ($\mu l\ l^{-1}$)	AA ($\mu l\ l^{-1}$)	EtOH ($\mu l\ l^{-1}$)
Control	1.25 a*	330.3 b	5.16 c	3.04 a	164.5 ab	4.60 a
Ethanol	1.00 a	444.2 a	6.23 b	4.60 a	228.0 a	5.20 a
LO_2-1d	1.82 a	390.1 ab	6.39 b	3.76 a	178.5 a	6.05 a
LO_2-7d	0.55 b	547.5 a	8.89 a	3.93 a	154.3 b	4.55 a

*as Tab.1

5.1.3. Scald development

After 6.5 months at 0°C in regular air storage plus 10 d at 20°C, LO_2-7d pretreated apples had the lowest superficial scald development, but those from the LO_2-1d pretreatment and also the ethanol-pretreated fruits exhibited high superficial scald scores (Table 4). The reduction in scald in the LO_2-7d pretreated apples was accompanied by reduced decay development. The ethanol and LO_2-1d pretreated apples showed more decay-enhancing damage than the control fruits (Table 4). The lower scald development in the LO_2-7d pretreated apples was associated with greater firmness and higher acidity after 6.5 months at 0°C (Table 4). The decay percentage, mainly caused by *Penicillium expansum,* increased during shelf life, especially on the ethanol and LO_2-1d pretreated fruits, and the decay was located on the peel, where it was affected by superficial scald (Table 4).

Table 4. Effect of various pretreatments on firmness, acidity, scald and decay development in Granny Smith Apples after 6.5 month at 0°C and 10 d at 20°C. Firmness results are means of 20 measurements (10 fruits). Acidity percentages are means of five fruits per treatment. Scald and decay are means of 12 nespacks (10 fruits/nespack).

Treatments	6.5 months at 0°C				+ 10 days at 20°C	
	Firmness (N)	Acidity (%)	Scald (index)	Decay (%)	Scald (index)	Decay (%)
Control	27.89 b*	0.84 c	2.83 b	2.22 b	8.37 a	3.84 b
Ethanol	27.56 b	0.95 b	4.44 a	7.18 a	8.38 a	19.92 a
LO_2-1d	28.16 b	0.92 b	5.97 a	4.76 a	7.64 a	17.17 a
LO_2-7d	33.65 a	1.03 a	0.60 c	0 c	3.05 b	5.81 b

*as Tab. 1

In the following season we conducted a similar experiment, but added another treatment: MCP at 0.5 ppm for 24 h at 20°C. MCP was very effective in reducing scald after both 6 and 8 months at 0°C and subsequent shelf life (Fig. 1). The non-chemical treatment, LO_2-7d was fairly effective: after 6 or 8 months at 0°C there was very little scald in this treatment, but after an additional 10 d of shelf life at 20°C some superficial scald developed (Fig. 1) and the browning intensity increased. The scald development in the control after 6 or 8 months at 0°C plus 10 d of shelf life at 20°C was similar to that in the ethanol and LO_2-1d treatments (Fig. 1). After 6 months at 0°C plus 10 days at 20°C the levels of decay were minor in all treatment, but after 8 months at 0°C plus 10 days at 20°C the ethanol- and (LO_2-1d)-pretreated fruits suffered from higher levels of decay (18%), while the control fruits had around 10% and those from the LO_2-7d and MCP pretreatments had very low decay development (Fig. 1).

5.1.4. Superficial scald and colour change

The superficial scald in Granny Smith apples after 6 months at 0°C in ambient air, starts from the flower end, which becomes browner while the stem end remains green. The Hue angles on the stem sides of fruits from the control, ethanol and LO_2-1d pretreatments were higher than those on the flower side, whereas in the LO_2-7d and MCP treatments the Hue angles on both sides were the same (Fig. 2). Similar patterns were found for the L* and C* values. The lightness and chroma values were significantly higher on the stem sides than in the flower sides of apples from the

were found for the L* and C* values. The lightness and chroma values were significantly higher on the stem sides than in the flower sides of apples from the control, ethanol and LO_2-1d treatments, but in the LO_2-7d and MCP treatments the L* and C* values were similar in both ends, which indicates that the colours of the two sides were similar, i.e., the fruits did not suffer from superficial scald (Figs. 2 and 3). In Fig. 3. it is clearly seen that after 6 months at 0°C followed by 10 d at 20°C, the MCP treated fruit did not suffer from any scald development and those from the LO_2-7d treatment suffered from only slight scald development, whereas, those from all the other treatments exhibited severe superficial scald development (Fig. 3).

5.1.5. Superficial scald and electrical conductivity (EC)

The increased browning in the flower end was correlated with greater ion leakage, as expressed as electrical conductivity (EC) *in situ*, after 6 months at 0°C. The apples from the control, ethanol and LO_2-1d pretreatments showed higher ECs in the flower end than in the stem end, whereas those from both the LO_2-7d and the MCP treatments had lower ECs than those from the other treatments and they exhibited similar values in the two sides (Table 5). Low values of EC in these fruits indicate better membrane integrity, and were correlated to a better texture and greener pulp tissue in the LO_2-7d and the MCP treated fruits.

Table 5. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on electrical conductivity ($\mu S.cm^{-1}$) in Granny Smith apples, measured *in situ* on two ends of the fruit – stem and flower ends – after 6 months at 0°C.

Treatments	Electrical conductivity ($\mu S.cm^{-1}$)	
	Stem end	Flower end
Control	63.44 a B*	85.57 a A
Ethanol	51.50 b B	71.26 b A
LO_2-1d	59.46 a B	88.22 a A
LO_2-7d	50.17 b A	56.52 c A
MCP	54.52 b A	52.76 c A

* Values within each column followed by the same low-case letter, and values within each row followed by the same capital letter, do not differ significantly according to Tukey's Test ($P < 0.05$).

5.1.6. Polyphenol oxidase (PPO) and peroxidase (POD) activities

The superficial scald development in the control, ethanol and LO_2-1d treatments of the Granny Smith apples was correlated with a higher activities of two oxidative enzymes – PPO and POD – after 6 months at 0°C (Fig. 4). The highest levels of both of these enzymes were found in the LO_2-1d treatment; the lowest level of PPO was in LO_2-7d treatment and that of POD in the MCP treatment. The low PPO and POD activities were associated with the reduced levels of scald in the LO_2-7d treatment, and with the complete freedom from scald in the MCP-treated fruits after long cold storage (Figs. 4 and 1, respectively).

5.1.7. Superficial scald and α-farnesene and conjugated trienols (CTs) levels

The level of α-farnesene was around three times as high as those of the CTs in all treatments after 8 months of storage at 0°C (Fig. 5). The levels of α-farnesene and CTs were significantly lower only in the MCP-treated fruits, whereas in the LO_2-7d treatment the levels were similar to those in the control (Fig. 5). The fruits from the ethanol and LO_2-1d pretreatments had the highest levels of α-farnesene, CT-258 and CT-259, and these were associated with greater scald development (Figs. 5 and 1, respectively).

5.1.8. Discussion

In Granny Smith apples low-O_2 stress for 7days (24 h at 20°C plus 6 days at 1°C) prior to cold storage, did not prevent the scald after 6.5 months at 0°C; on the contrary, it increased the damage. The inability of (LO_2-1d)-pretreated apples to maintain low AA and ethanol levels during shelf life after 1 month at 0°C indicates that the tissue was damaged, which led to increased scald development later (Tables 1 and 4). Also Ghahramani and Scott (1998) showed that low-O_2 stress at 0°C for 2-4 weeks was not effective in reducing scald development, but that only low-O_2 stress that was applied continuously for long periods of 8-16 weeks was effective. In addition, it has been shown that initial low-O_2 stress (ILOS) followed by CA storage was effective in controlling scald in apples (Wang and Dilley, 2001; Zanella, 2003).

In various subtropical fruits short periods of low-O2 stress or high CO2 can reduce CI symptoms, mainly because of the reduction in ethylene production (Pesis, 2005). However, in apples longer periods are needed and low-O_2 stress given in a flow system for 12 days at 20°C was the most effective in reducing scald (Ghahramani and Scott, 1998). In the present study the (LO_2-7d)-pretreated apples had the lowest ethylene and the highest AA and ethanol levels following 6.5 months of storage at 0°C (Table 3), and this could explain the lowest scald development (Table 4). The effect of AA in inhibiting ethylene production was more pronounced than that of ethanol, as has been shown in several other fruits, including grape, tomato, mango, avocado and banana (Pesis, 2005). It is already known that the synthesis of α-farnesene is mediated by ethylene (Watkins et al., 1993; Ju and Curry, 2000). Our findings of reduced α-farnesene and CTs in MCP-treated fruits are consistent with the results of other workers who found that inhibition of ethylene by MCP dramatically reduced the levels of α-farnesene and CTs, which led to reductions in scald development (Fan et al., 1999; Rupasinghe et al., 2000; Watkins et al., 2000a; Shaham et al., 2003; Zanella, 2003; Haines et al., 2005).

The activities of the oxidative enzymes PPO and POD showed that fruits that were pretreated with LO_2-7d or MCP exhibited lower activity levels of these two enzymes (Fig. 4), whereas those from the LO_2-1d pretreatment had the highest levels, which may indicate more damage to the membranes, as indicated by the electrical conductivity measurements (Fig. 4 vs. Table 5). Bauchot et al. (1999) showed that the levels of PPO expression were higher in peel from apples that suffered from scald, but they concluded that the PPO expression was not necessarily associated with the initial development of the scald symptoms. CI symptoms in avocado, expressed as mesocarp discoloration, were found to be related to increases in PPO and POD activities and to ion leakage (Hershkovitz et al., 2005). Also in apples that suffer from superficial scald the peel browning is considered as a CI symptom and it is associated with enhanced PPO and POD activities and greater membrane degradation. Our present results are in agreement with those of Hodges et al. (2004), who concluded that suboptimal

temperature storage caused increases in pitting, flesh browning, POD activity and membrane degradation, and decreases in antioxidant contents.

5.1.9. Conclusion

In the present study, the effectiveness of MCP at 0.5 μl l^{-1} in reduction of superficial scald in apples was better than that of LO_2-7d (Figs. 1 and 3). The MCP-treated fruits did not suffer from scald, also after removal to 10 days of shelf life after 6 and 8 months at 0°C, whereas those from the LO_2-7d pretreatment exhibited some scald development. MCP is a powerful compound that inhibits ethylene activity, and its effectiveness in reducing scald by reduction in α-farnesene and CTs has already been proven (Rupasinghe et al., 2000; Watkins et al., 2000a). On the other hand, warming apples of various cultivars for 0-9 days at 20°C prior to cold storage was not effective in reducing scald (Watkins et al., 2000b). In our present study the combination of low O_2 and room temperature of 20°C for 7 days was effective in reducing scald (Table 4, Figs.1 and 3), whereas low-O_2 treatment for 1 d at 20°C plus 6 d at 1°C, was not effective in reducing scald, probably because there was less production of anaerobic metabolites (Tables 1 and 2).

It seems that both methods – exposure to 1-MCP and low-O_2 stress – were effective in reducing scald development in Granny Smith apples after 6-8 months at 0°C. Although MCP was more effective, the low-O_2 stress provides a simple non-chemical means that could be suitable for reducing superficial scald development in Granny Smith apples for the organic fruit markets. In addition, the simplicity, low cost and efficacy of the treatment suggest that it is better suited to developing countries.

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Fig 1. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on superficial scald (A, B) and decay development (C), in Granny Smith apples after 6 and 8 months at 0°C plus 10 days at 20°C.

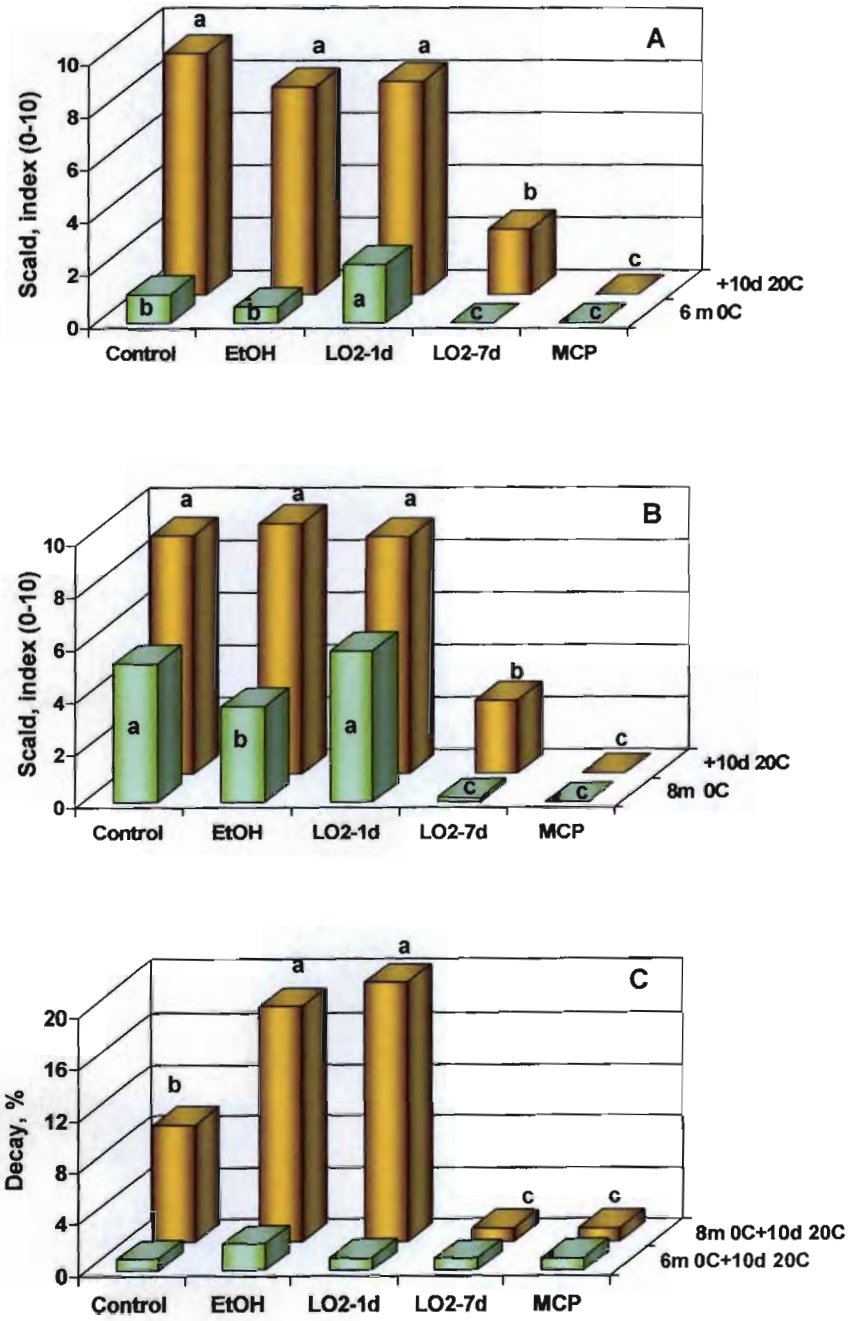


Fig. 2. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on lightness (L*), chroma (C*), and hue angle (H°) in Granny Smith apples on two ends of the fruit – stem and flower ends – after 6 months at 0°C.

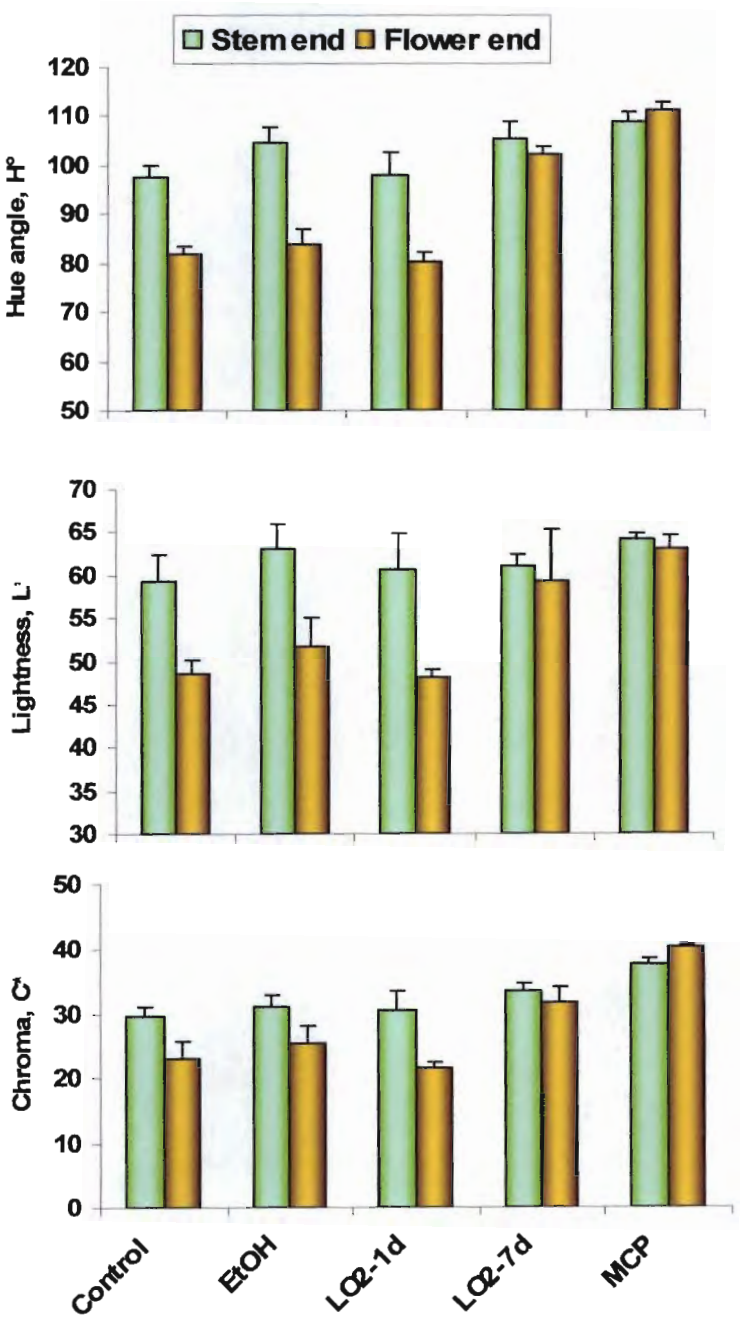


Fig. 3. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on fruit appearance of Granny Smith apples after 6 months at 0°C and 10 days at 20°C.

Fig. 4. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on polyphenol oxidase (PPO) and peroxidase (POD) activities in Granny Smith apples after 6 months at 0°C.

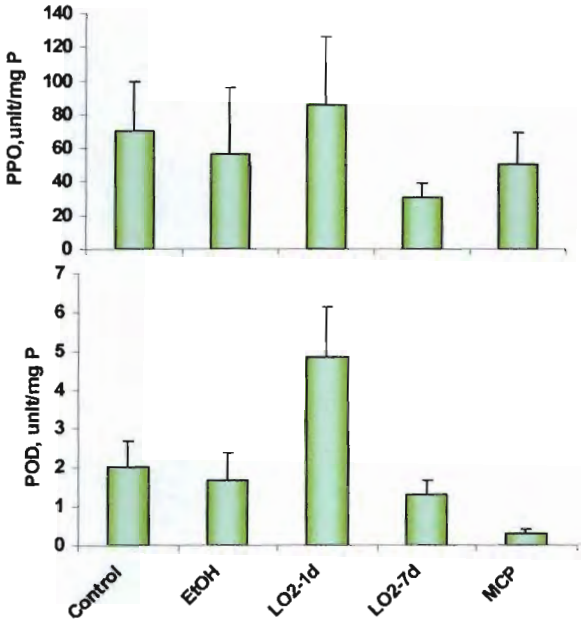
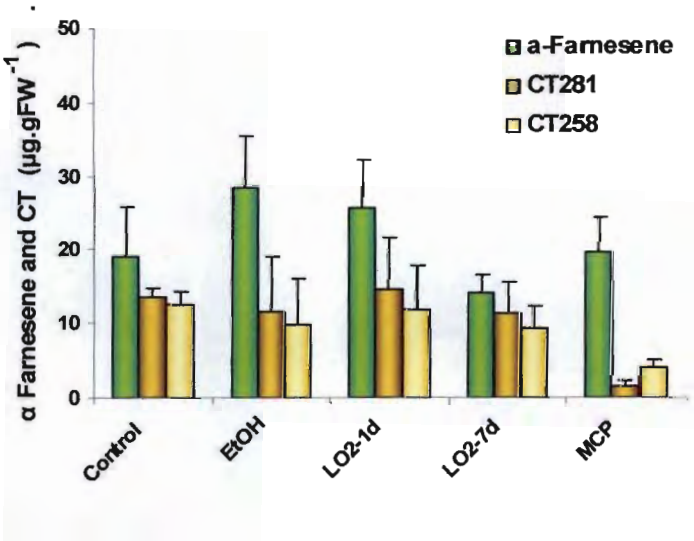


Fig.5. Effects of ethanol vapour (EtOH), LO_2-1d, LO_2-7d, and MCP pretreatments prior cold storage, on α-farnesesne and conjugated trienols (CTs) levels in Granny Smith apples after 6 months at 0°C.



5.2. Use of ethanol to control decay in table grapes during storage

Several studies in the last decade have shown that ethanol can retard decay development. Ethanol treatment reduced postharvest decay of citrus and stonefruit. Ethanol at 20 or 45% entirely suppressed the linear growth of *B. cinerea* and *Monilia fructicola* in inoculated peaches (El-Sheikh Aly et al., 2000). Recently it was shown that dipping grapes in 30 to 50% ethanol prior to packaging inhibited berry decay as well as or better than SO_2 released from generator pads (Lichter et al., 2002; Karabulut et al., 2004; Chervin et al. 2005). Decay control was feasible for a cold storage period of 4 to 5 weeks and sometimes longer. Ethanol did not impair the appearance of the bunch, and ethanol-treated berries had higher organoleptic scores than those treated with SO_2 (Lichter et al., 2002). In this study, in addition to pre washing the grapes with ethanol (35% solution), we have tested the application of ethanol during storage by continuous evaporation or in combination with reduced amount of slow released pads of SO_2 to improve grape quality.

5.2.1. Fruits and treatments

'Superior' grapes were harvested in Israel with an total soluble solids (TSS) of 14.7±0.32 %, TA of 0.45 ±0.03 % and berry size of 6.5±0.5 g. The grapes bunches were pre-washed at the same day of harvesting with ethanol 35% or remained unwashed as described by Lichter et al., (2002). The bunches were dried and placed in 1.5 kg punnets for a day at 0C. A day later, the punnets were enclosed in polyethylene (PE) bags (45 μM thickness) in the presence of ethanol or sodium metabisulfite: ethanol 4 or 8ml in 15 ml polypropylene tubes were placed in punnets with 1.5 kg grapes 'Superior' with a paper wick; SO_2 pads 0.3 or 0.6gr were placed above a silk paper in each punnet. Control grapes were packed in PE bags without any chemicals and sealed with a rubber band 24 h after the beginning of cold storage at 0°C. Each treatment had four replicates. Grapes were stored for 6 wks at 1°C + 3d at 20°C.

5.2.2. Volatiles accumulation in grape packages

The accumulation of AA and ethanol in the atmosphere surrounding the grapes during storage depended on the type of ethanol application. The punnets containing 1.5 kg of 'Superior' grapes had appreciable levels of acetaldehyde and ethanol during the 6 weeks of storage at 0°C in the treatments of 4 and 8 ml of ethanol with wicks (Fig. 6). Release of ethanol into the package declined over time in the 4 ml treatment, while in the 8 ml package there was some decline during the first 3 weeks and then the level remained steady at an average level of 700±117 ppm of ethanol. AA levels also declined in the 4 ml package from 25 days onwards, while they remained steady in the 8 ml package. Although ethanol was low in the packages that had received a pre-storage 35% ethanol dip, there was an appreciable level of AA in this treatment at the beginning of storage that gradually disappeared during the storage period. The acetaldehyde detected in the headspace of the punnets after dipping in ethanol could result from conversion of ethanol which was absorbed by the berry to acetaldehyde. The levels of ethanol in the head space of unwashed grapes which were treated with 8 ml ethanol was much higher than that of washed grapes (Fig. 6).

Ethanol and acetaldehyde are precursors of natural aroma compounds, and in many fruits they accumulate naturally during ripening (Knee and Hatfield, 1981). External treatment with ethanol at verasion enhanced grape maturation (Chervin et al., 2001). This occurred both in table and in wine grapes (El Kereamy et al., 2002). This

preharvest treatment enhanced the accumulation of aroma compounds. This positive effect of ethanol is in addition to the antifungal effects that were tested here.

5.2.3. CO_2 and O_2 levels in grape packages

The polyethylene bags that enclosed the 1.5 kg punnets of 'Superior' grapes prevented water loss but did not develop an appreciable level of modified atmosphere (Fig. 7). Unwashed control grapes had the highest level of CO_2 in the bags after 7 weeks which was close to 2%, while the ethanol dipped grapes had lower levels of CO_2 of 1.4% (Fig. 7). On the other hand the oxygen concentrations in the punnets of unwashed control fruit was lower (around 15%) than that of washed fruit (around 18%). The difference in CO_2 and O_2 between pre-washed ethanol and unwashed clusters was probably caused because of fungal contamination in the unwashed grapes.

5.2.4. Chemical contents in grape juice after 6 weeks at 1C

Overall, the entire treatments the levels of sugar expressed as TSS and acidity were lower in unwashed grapes compare to pre washed ethanol grape (Fig. 8). The reason for this again, is that the pre washed grapes were less contaminated with fungus, so the respiration was lower (as shown by lower CO_2) and less sugar and acid are metabolized in the respiration processes.

The levels of endogenous AA and ethanol in the various treatments was significantly different. Grapes that were stored with 4 or 8 ml ethanol for 6 weeks at 0°C, had much higher levels of AA and ethanol than all other treatment (Fig. 9).

5.2.5. Grape quality after 6 weeks at 1C

After 6 weeks of storage and additional 3 days at 20°C, there were significant differences in the decay incidence among treatments (Table 6). The control grapes were almost all decayed, while those with an SO_2 pad showed no decay. Both the 4 and 8 ml ethanol wick treatments also completely controlled decay, while the ethanol dip alone was less effective (Fig. 10). This was expected, since previous work has shown that the prestorage ethanol dip does not protect against reinfection, or against spores that may already have penetrated into the fruit (Lichter et al., 2002, 2005). The treatments that maintained a level of ethanol inside the package gave protection against decay development throughout the storage period. Freshness of the bunches and desiccation of stems and pedicels was similar in all the treatments. However, the ethanol vapor treatments caused minor berry browning which was not apparent in either the untreated control or the SO_2 pad.

5.2.6. Conclusion

The ethanol vapor treatments have the advantage over ethanol dips in that they confer extended protection, similar to the SO_2 pad. This allows the control of latent infections that may emerge during storage, and which the prestorage treatment cannot prevent. Our results are in agreement with those of Chervin et al., (2005).

The adverse effects of the ethanol treatment in increasing stem desiccation and berry browning may be due to the acetaldehyde that is generated from ethanol. The treatments that caused the highest level of berry browning were those with the highest levels of acetaldehyde in the atmosphere. Acetaldehyde has been shown to cause browning reactions in a number of fruits and vegetables, including apple, cherry,

lettuce and tomato (Pesis, 2005). Alternatively, continuous ethanol application may dry rachis by dissolving its epicuticular wax protection.

Table 6: Quality attributes of 'Superior' grapes unwashed and pre-washed with ethanol 50% after storage for 6 weeks at 0°C and then held for 3 days at 20°C. All indices scales were in ascending scale from 1 to 5.

Treat	Firmness index	Decay index	Desiccation index		Browning index
			Stems	Pedicels	
Unwashed					
Control	1.82 a*	4.75 a	2.25 a	2.52 a	1.00 b
0.3 SO_2	1.77 a	1.00 c	1.95 b	2.37 a	1.00 b
0.6 SO_2	1.75 a	1.00 c	1.97 b	1.97 b	1.00 b
4 ml EtOH	1.75 a	1.25 b	2.12 a	2.22 ab	1.37 a
8 ml EtOH	1.77 a	1.50 b	2.35 a	2.40 a	1.75 a
Washed					
Control	1.72 a	1.37 a	2.37 a	2.80 a	3.00 a
0.3 SO_2	1.70 a	1.00 b	2.20 ab	2.75 a	1.37 c
0.6 SO_2	1.70 a	1.00 b	2.02 b	2.07 b	1.00 c
4 ml EtOH	1.77 a	1.00 b	2.40 a	2.55 ab	2.25 b
8 ml EtOH	1.80 a	1.00 b	2.50 a	2.90 a	1.75 b

*as Tab. 1

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Fig. 6. Effect of various treatments on acetaldehyde (AA) and ethanol (EtOH) concentrations (ppm) inside bags with 'Superior' table grapes unwashed and washed with ethanol prior storage, during 6 weeks at 1°C.

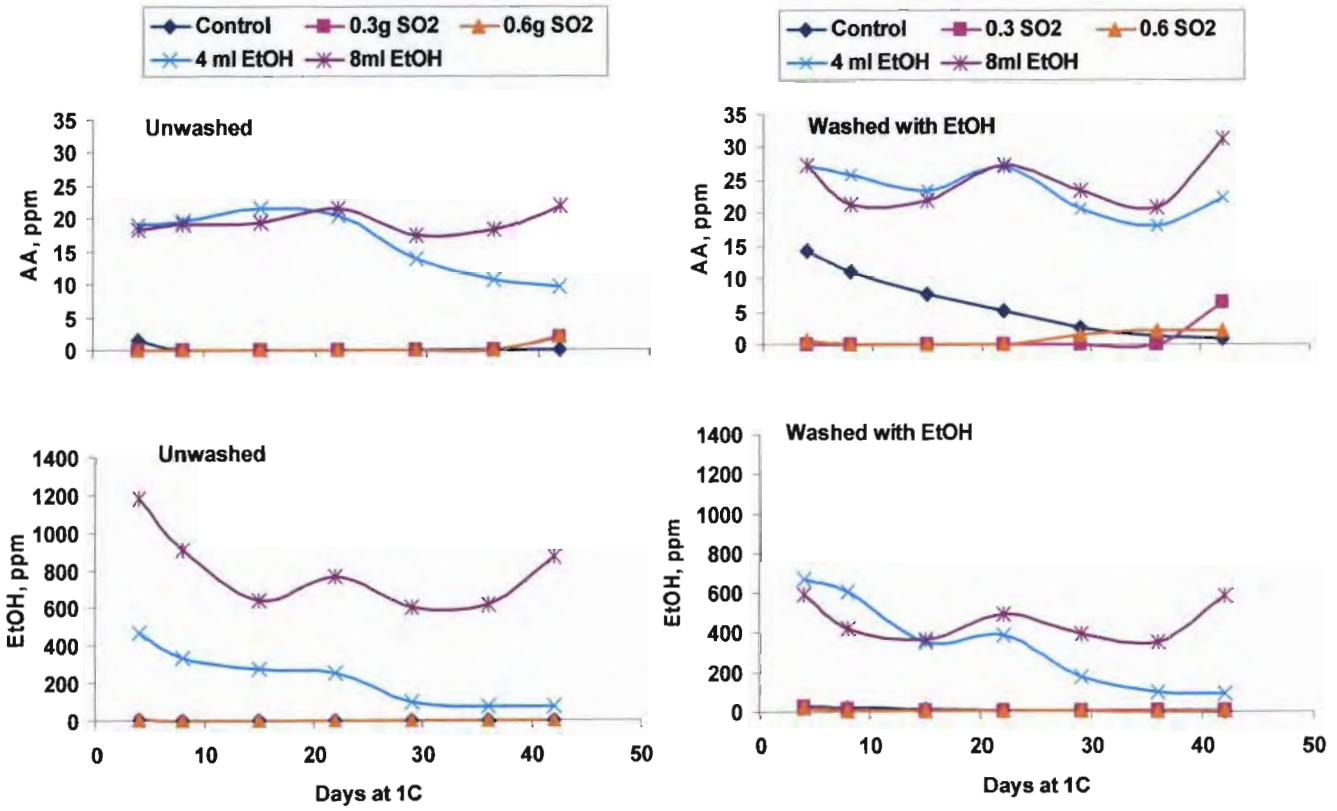


Fig.7. Effect of various treatments on CO_2 and O_2 concentrations (%) inside bags with 'Superior' table grapes unwashed and washed with ethanol prior storage, during 6 weeks at 1°C.

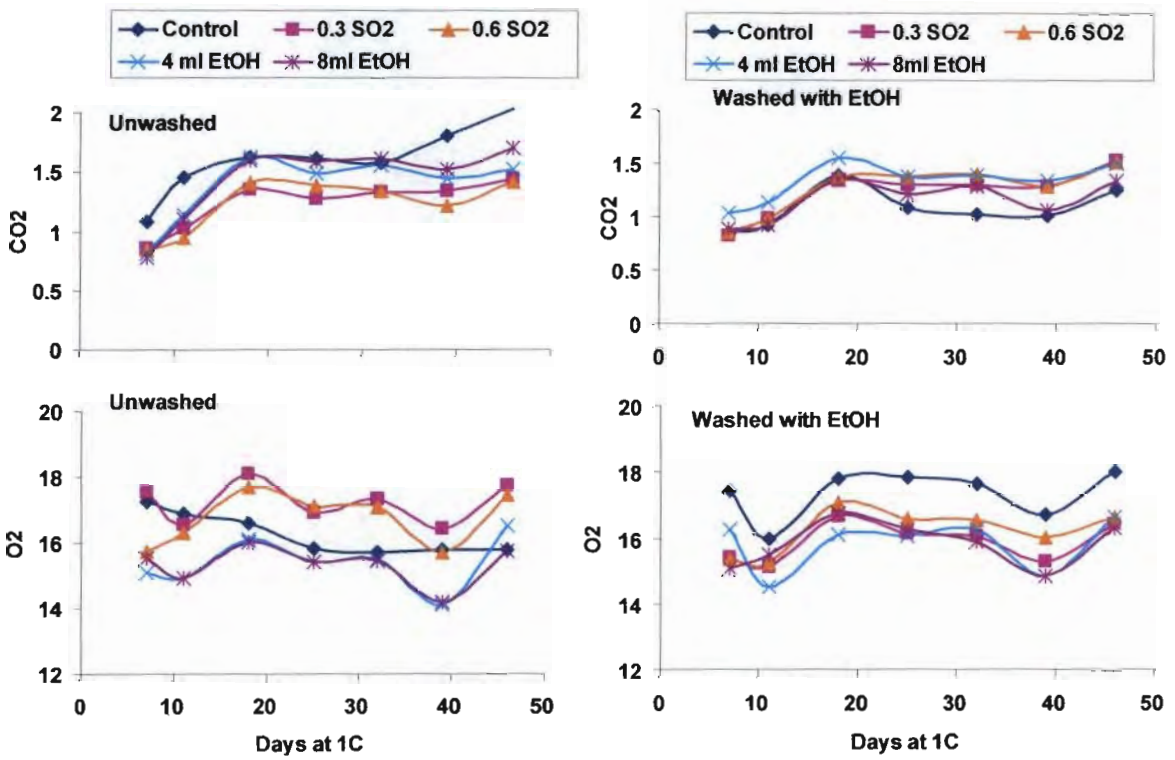


Fig. 8. Effect of various treatments, on TSS and acidity levels in the juice of 'Superior' grape unwashed and washed with ethanol prior storage, after 6 wks at 1C

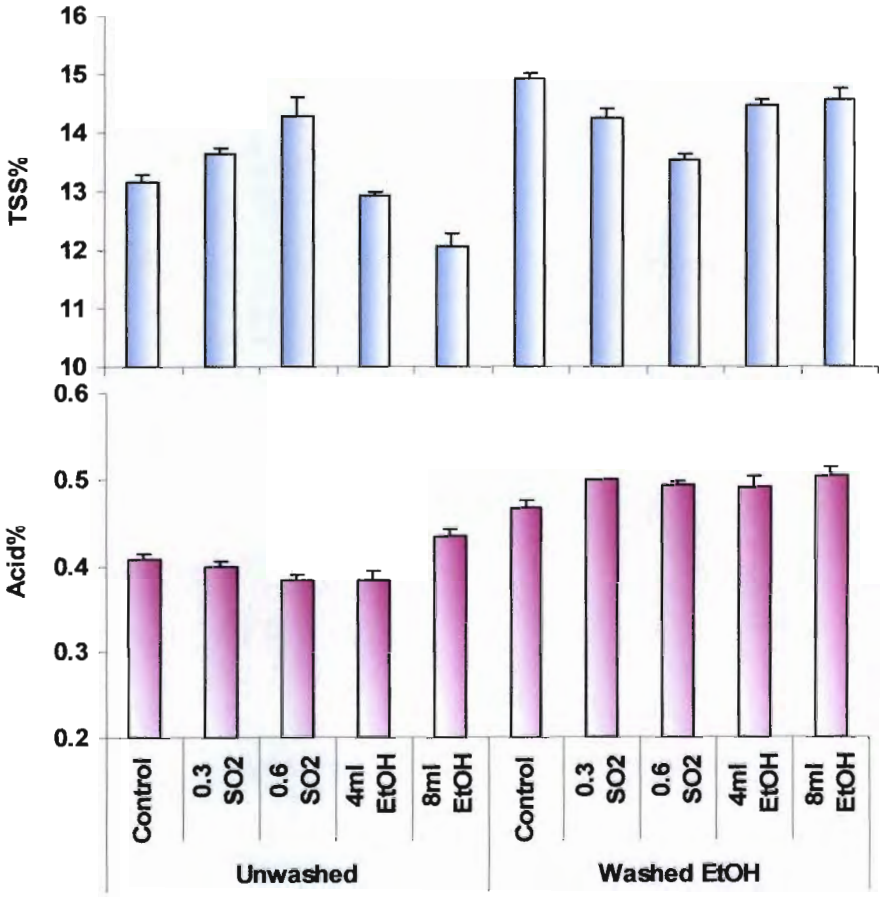


Fig. 9. Effect of various treatments, on acetaldehyde (AA) and ethanol (EtOH) endogenous levels in the juice of 'Superior' grape, after 6 wks at 1C.

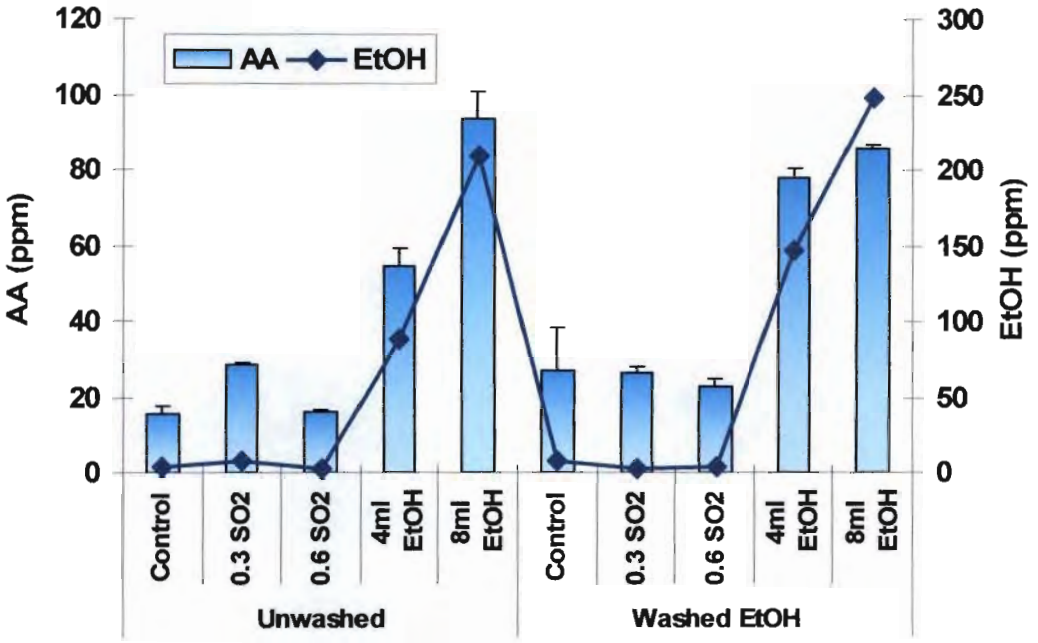
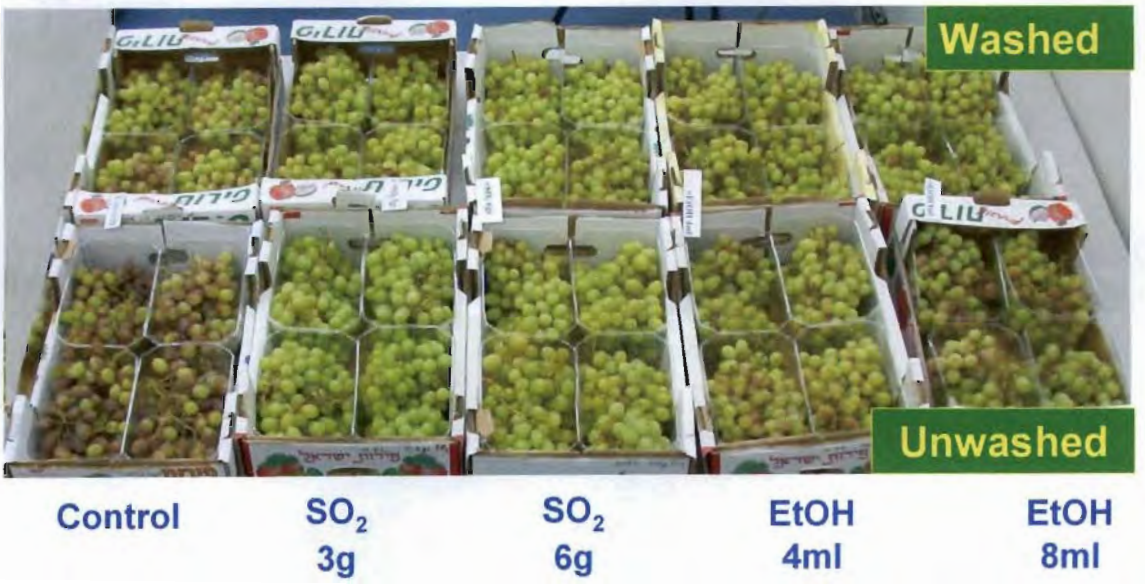


Fig. 10._ Effect of various treatments, on 'Superior' grape appearance after 6 wks at 1C



5.3. Alternative methods to SO_2 for keeping quality of grape cv. Taifi rosoviy for long storage

5.3.1. Effect of various bioactive compounds

In the lab in Kazakhstan, innovative biological materials were developed with antioxidative activity that was extracted from natural resources. These compounds were applied to various fruit including grapes to improve their quality in storage. The grape of the variety 'Taifi rosoviy' was harvested in Kazakhstan at optimal consumer maturity and stored in boxes for long storage at 0°C. The grape clusters were treated with different bioactive preparations by dipping the clusters in various solutions as shown in Table 7.

The changes of berry quality appeared after 3 month of storage. The treatment of grape berries by various antioxidants (GX, KF and ethanol) disinfected the grapes and promoted the best keeping quality of clusters, less shatter of berries and delayed skin browning (Table 7).

Table 7. The influence of the preparations on grape keeping quality of the variety 'Taifi rosoviy' after 6 month of storage at 0°C.

Variants	Decay, %	Shattered, %	Total loss, %
Control	13,0	9,5	22,5
GX 0,01%	8,2	8,3	16,5
0,03%	8,2	7,1	15,3
0,09%	10,0	7,0	17,0
KF 0,1+ EtOH 25%	4,8	7,3	12,1
KF 0,01	9,8	7,4	17,2
Ethanol 25%	5,0	7,5	12,5
$CaCl_2$ 6%	6,0	8,5	14,5

The storage period of 'Taifi rosoviy' grapes was extremely long (6 month) and could be extended to 9 months ??. Grapes treated by the antioxidant GX with concentration of 0,03% seemed to be better then higher or lower doses. The treatment by the GX at this concentration reduced berry browning significantly (not shown). The best treatment was the combination of KF 0.1% + Ethanol 25% to retard rot development and natural losses (shattered berry) (Table 7). Dipping the grape bunches in ethanol 25% or $CaCl_2$ 6% solution without addition of bioactive compounds also show good results in reducing losses of 'Taifi rosoviy' grapes (Table 7).

5.3.2. Application of Intermittent CO_2 atmosphere to grapes

High concentrations of CO_2 (40-50%) had negative influenced on grapes. Keeping the berries in this condition for 2 hours led to skin browning and skin separation from the flesh. Fumigation, conducted twice during two hours every 10-15 days led to berry browning and berry shatter of 14%.

Thus, it was established that high concentration of CO_2 (40-50%) is to high for treating grapes. Also fumigation with 30% CO_2 during 2 hours every 10-15 days was not effective. After 3 treatments grape berries became brown and the amount of shatter was 14%.

The introduction of 20% CO_2 into packages with grape for three times every 10 days (2 hours each time) demonstrated that this method may be a useful alternative to the traditional fumigation by sulfur dioxide. The grape quality parameters were better than the control. The amount of rotted and shattered berries of grape variety 'Taifi rozoviy' after 90 days at 0°C was lower in the CO_2- treated grapes (Table 8). In this treatment the development of fungal rot is suppressed and in addition the physiological losses are significantly reduced. The total losses in control under regular air were 2 times higher than in the CO_2 – treated grapes.

Table 8. Effect of intermittent 20% CO_2 application on grape cv. Taifi rozoviy, quality after storage in hermetic packages for 90 days at 0C.

Variants	Standard, %	Decay, %	Shattered, %	Total losses, %
Control	89,2	6,7	4,1	10,8
CO_2 - treated	94,4	3,8	1,8	5,6

6. Impact Relevance and Technology Transfer:

In this project we studied and applied several innovative and relatively simple technologies that can be useful also in developing countries. All the knowledge that accumulated from this project in the Volcani Center was effectively transferred to the research institute in Kazakhstan via the three trainees that were involved in developing the new technologies.

The first technology was coating fruit with various organic waxes to improve fruit quality (described in the first and second annual reports). We have shown success in extending shelf life of various fruits including melon and apple.

The second technology is application of the ethylene inhibitor, 1-MCP to prevent scald in apples. 1-MCP is an innovative molecule that gave excellent results in avoiding scald in apples, and doing it in Kazakhstan can contribute a lot to the ability of Kazakhstan to export apples.

The third technology is to use low O_2 treatment in order to avoid scald in apples. This method is less effective than 1-MCP application. However, it is not a chemical and it can be applied even for organic fruit. Moreover, the simplicity, low cost and efficacy of the treatment can be applied very easily to developing countries.

The forth technology includes application of ethanol solution, as prestorage treatment and application of ethanol vapors to avoid decay development in grapes as alternative to the use of SO_2.

7. Project Activities/Outputs:

List of meetings attended:

Dr. Tamara Uryupina visited Israel on September 2003. During her visit we spent time in the lab and went together to the agriculture exhibition (Tel Aviv) and packinghouse (North of Israel).

On September 2004 Dr. Edna Pesis and Mr. Oleg Feygenberg visited the institute of apple and grape in Almaty, Kazakhstan. This visit was very efficient: we visited the

various labs and gave a talk about our research in Israel. In addition we visited the orchards where many new cultivars of apples are grown and the tissue culture labs where the genetic stock material of apples is maintained.

List of trainees:

Mrs. Tamara Nikitenko
Ph.D. student, Inst. of Apples and Grapes, Union "Almaly", Alma-Ata, Republic of Kazkhstan. Duration of training in the lab in Israel: Feb. 2003 - Oct. 2003.

Mrs. Oxana Gadiyeva
Ph.D. student, Inst. of Apples and Grapes, Union "Almaly", Alma-Ata, Republic of Kazkhstan. Duration of training in the lab in Israel: April 2004 – Dec. 2004.

Mr. Ivan Antilofyev
Ph.D. student, Inst. of Apples and Grapes, Union "Almaly", Alma-Ata, Republic of Kazkhstan, Duration of training in the lab in Israel: Feb. 2005 – Aug. 2005.

8. Project productivity:

Lectures in International Symposium

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