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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 last year, we have developed a new wax of colloidal solution based on beeswax. This edible coating is prepared without using solvents and it includes only organic components such as: oils, alcohols, salts, phospholipids and natural vitamins. We tried this coating on various fruit commodities including apple, melon and mango. Coating melons reduced water loss and did not cause off-flavors accumulation. Moreover, the levels of endogenous CO_2, acetaldehyde (AA), ethanol and ethyl acetate, were lower than in fruit waxed with synthetic wax and the taste panel preferred this fruit. 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 functional disorders. Also in the coated apple there was less accumulation of AA and ethanol after 6 months of storage which indicates on better quality.

Dipping grape clusters in 25-35% ethanol solution gives sterilization effect and keeps better quality of grape clusters cv. Taifi Rosoviy, less shattering of berries and delay of skin browning. Dipping grape bunches cv. Thompson seedless in ethanol solution and then storing them 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 AA. Application of AA vapors was very effective in prevention development of fungi *in vitro*.

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 those improve the quality of the fruits.
- To study the influence of antiseptic postharvest treatment to grape clusters of ethanol or calcium chloride solution on quality.
- To study the role of ethanol and AA in fungus growth inhibition.

B. Research accomplishments

In Israel and Kazakhstan, we developed a simple and inexpensive method for extending shelf life of fruits by the use of edible coatings that improve the mechanical handling properties and delay their ripening. In Israel, a new organic wax based on beeswax was invented. This wax enrobes fresh fruit and vegetable and can be suitable even to organic fruit. In Kazakhstan, a new fruit coating, based on mono and triglycerides integrated with oil was developed. This fruit coating was improved by adding to it bioactive materials that have antioxidant activity. These two types of coating serve as good semi-permeable barriers to gases and water vapors. In addition it maintained fruit aroma and taste and prevented decay development.

Coating fruit In Israel

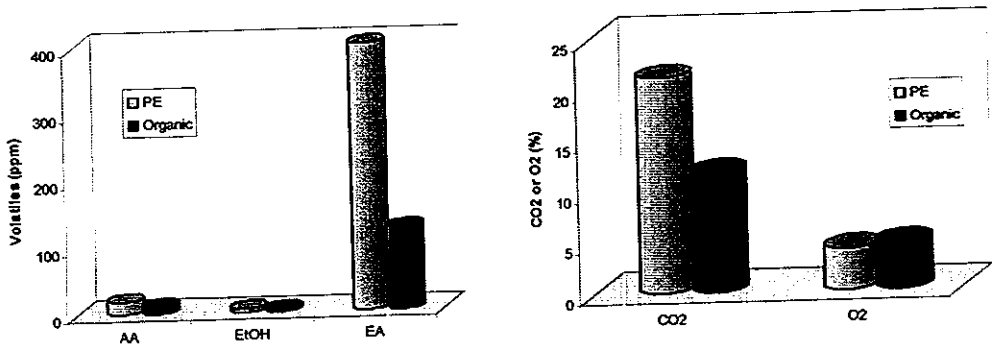
We develop new organic wax, which is a colloidal solution based on beeswax. The organic wax includes only organic components such as: oils, alcohols, salts, phospholipids and natural vitamins. The organic wax was applied prior to storage to melon cv. Galia. The effectiveness of this organic wax was examined against regular synthetic wax based on polyethylene (PE). Generally, the melon that was treated with the organic wax maintained a better quality than the commercial fruit that was treated with PE wax after 2 weeks at 5C and additional 3 days at 17C (Table 1). The fruit that was coated with the organic wax maintained a better firmness expressed in Newtons, and a lower weight loss. At this stage, there was no significant difference in decay development and sugar levels expressed as total soluble solids (TSS) (Table 1). However, keeping the fruit for longer time at shelf life, 2 weeks at 17C, after 2 weeks at 5C revealed that the fruit that were treated with organic wax had lower decay percentage (36%) compare to almost 100% decay in the commercial fruits.

Table 1: Effect of various waxing in Melon cv. Galia on quality indices after 2 weeks at 5C and 3 days at 20C.
ab, Mean separation in columns by Duncan's multiple range test, 5% level.

Wax	Appearance 1-5	Weight loss (%)	Firmness N	TSS %	Decay %
PE wax	3.4 b	1.94 a	48.3 b	10.7	0
Organic wax	4.0 a	1.76 b	69.5 a	10.3	4.2

The anatomical structure of the melon has advantage that gas samples can be taken from the fruit cavity showing us the real endogenous atmosphere in the fruit. It seems that the organic wax enables good gas exchange via the peel as was shown by the interior atmosphere in the fruit cavity (Fig. 1). Moreover, treating the melon cv. Galia with the regular PE wax caused a fermentative process inside the fruit which brought to the production of off-flavors, while treating with the organic wax there was a much lower amount of fermentative products including acetaldehyde (AA), ethanol (EtOH) and ethyl acetate (EA) (Fig. 1). From this data we can conclude that the synthetic wax (which is the commercial one) is less permeable to gases, which caused the accumulation of more fermentative products.

Fig. 1. Effect of various waxing in Melon cv. Galia on the levels of endogenous gases: CO_2, O_2 acetaldehyde (AA), ethanol (EtOH) and ethyl acetate (EA) in the melon cavity after 2 weeks at 5C and additional 3 days at 17C.



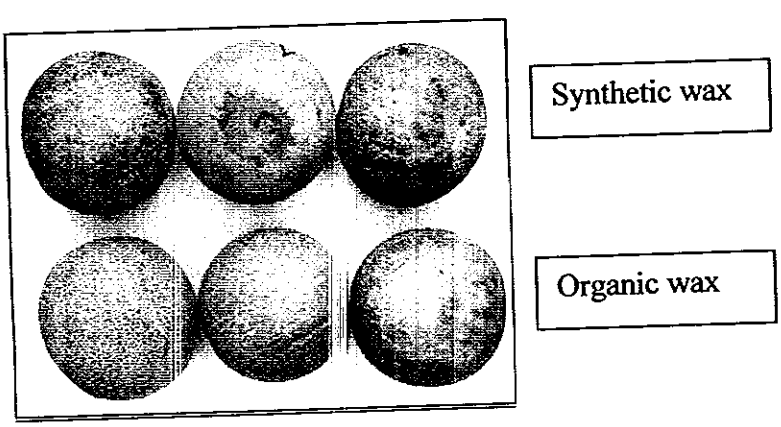
In addition to the measurement of the endogenous gases we preformed a taste exam based on the triangle exam (Kramer & Twigg, 1966). In this test, Panelists were required to identify which sample is different from a set of 3 samples that includes two identical samples. In the case of melon, 21 panelists recognized the odd from a total 27 panelists. This result is significant at 0.001% level. Moreover, 85% of them preferred the fruit that was treated with organic wax and gave it higher grades than the commercial one that was waxed with synthetic wax (Table 2).

Table 2: Effect of various waxing in Melon cv. Galia on taste preference after 2 weeks at 5C and 3 days at 20C. (data are average of 27 panelists ± SE.

Treatment	Taste grade (1-9)	Taste preference (%)
PE wax	4.46 ± 1.54	15
Organic wax	7.05 ± 1.49	85
Organic wax	6.88 ± 1.45	

The melon that were coated with the organic wax looked much better than the commercial fruit that was coated with synthetic wax (Picture 1).

Picture 1: Effect of various waxing in Melon cv. Galia on fruit appearance after 2 weeks at 5C and 3 days at 20C. Upper row – Synthetic PE wax; Lower row – Organic wax



Coating fruit in Kazakhstan

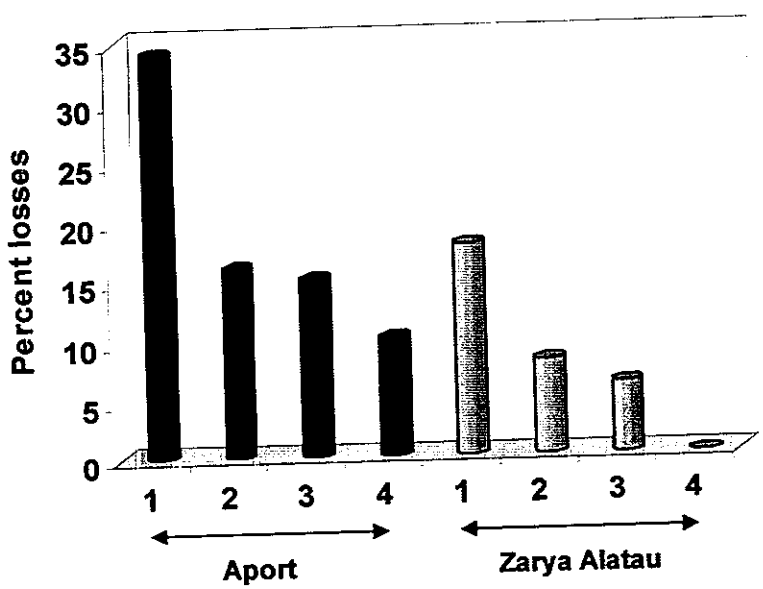
The apple fruits of the varieties Aport and Zarya Alatau, regionalized in Almaty region, were used for the study.

The following preparations were examined as film-forming coatings:

1. Monoglyceride (MG)
2. GLES is synthesized by thermal interaction of triglycerides and oil in the presence of catalyst.
3. Mineral coating by a water solution of 4% calcium chloride.

All the film-forming coatings are well soluble in water. The best results in experiments with postharvest treatments of Aport apples were obtained in the variant with the use of the preparation "MG". The losses from damages were 3.5 times lower in this variant than in control. The coating of fruits by film-forming preparations "GLES" and calcium chloride reduces the losses by 2 times (Fig. 2).

Fig. 2. The effect of various coating on percent of apple fruit losses in cvs. Aport and Zarya Alatau after 6 months of storage at 0C.
Film forming coatings: 1 – Uncoated; 2- Calcium chloride; 3- GLES; 4-MG



In experiments with apple fruits Zarya Alatau, treated by the preparation "MG", fruit losses was zero (Fig 2). Looking at the biochemical changes occurred in the fruit before storage and after storage, it is obvious that the sugars, vitamin C and protopectin are reduced dramatically, while acetaldehyde (AA) and ethanol contents increase during aging (Table 3). However, the film-forming coatings with MG or calcium chloride delay fruit senescence. The levels of AA and ethanol are lower in the treated fruit compared to the control after 6 months of storage. Higher levels of AA and ethanol are good indicators for fruit senescence (Table 3).

Table 3: Effect of MG and calcium chloride solution on biochemical composition of apple fruit Zarya Alatau (%) after 6 months of storage at 0C.

Experimental variants	Dry sub-stance	Suc-rose	Sum of sugars	Aci-dity	Vita-min C	Proto-pectin	Sum of pectin	Ethanol mg%	AA mg%
Control before storage	11,4	2,9	11,2	0,9	9,9	0,76	1,2	11,0	0,57
After storage	12,0	0,3	8,6	0,7	5,9	0,40	1,1	57,0	1,23
"MG"	11,2	1,6	9,8	0,54	7,2	0,65	1,2	15,0	0,70
Calcium chloride	11,0	1,1	9,0	0,7	6,7	0,57	1,1	17,0	0,72

Also application of various natural vegetative antioxidants (Vitoran, Bitonin) and Synthetic preparations, possessing antioxidant activity (Bio) and analogue of the synthetic fungicide benomile (Azocene) reduced the levels of AA and ethanol after 6 months of storage at 0C (Table 4).

Table 4: The content of ethanol and acetaldehyde in apple fruits Aport after 6 months at 0C.

Postharvest treatment	Ethanol, mg%	Acetaldehyde, mg%
Control water	74,0	1,98
Vitoron	41,5	1,22
Bitonin	32,0	1,13
Bio	57,0	1,54
Azocene	68,0	1,42

Again, as in Zarya Alatau also in Aport apple, alcohol and acetaldehyde can be good indices to determine which of the postharvest treatments was more effective in delaying the processes of fruit aging. The higher the levels of these volatiles - the more senescent is the fruit. This conclusion is in agreement with the results previously shown in various cultivars of apples and pears (Smagula and Bramalage, 1977).

In order to increase the effectiveness of the film coating various bioactive substances were added. In addition to the compounds shown in Table 4, we added another 2 synthetic origin water-soluble antioxidants - Santochine, Phenosan.

1. GLES + Santochine.
2. MG + Vitoran.
3. MG + Bitonin.
4. MG + Vitoran + azocene.
5. GLES + phenosan + azocene.
6. CaCl2 + phenosan + azocene.

All treatments were done by dipping the fruit in the solutions. All experimental fruit samples were stored in cool chambers under optimal conditions (Temp. = 0C, relative air humidity 85-90) during 6-7 months.

As it is seen from Table 5 surface-active preparations (combination of film-forming coatings with antioxidants and fungicides) enhanced considerably the fruit keeping quality of both apple varieties.

Table 5: The influence of surface-active preparations on percentage of apple fruits preservation of two cultivars after 6 months of storage at 0C.

Experimental variants	cv. Aport Preservation (%)	cv. Zarya Alatau Preservation (%)
Control (water)	66,6	82,2
GLES + santochine	87,0	98,0
MG + Vitoron	93,0	100,0
MG + Bitonin	94,0	100,0
MG + Vitoron + azocene	96,5	100,0
GLES + santochine + azocene	92,0	100,0
Calcium chloride + phenosan+azocene	90,0	97,2

In conclusion: the use of preparation mixtures possessing different action spectrums, for postharvest treatments, will allow to reduce apple fruit losses from attacks by pathogenic microorganisms and to prevent functional disorders.

Ethanol treatments to grape for reduction of decay

Grape clusters cv. Taifi Rosoviy grown in Kazakhstan, treated with 6% $CaCl_2$ and 25% ethanol solution, were distinguished by good trade state and by higher output of standard clusters (Table 6). The total losses for the storage period in the control were 21%, in variants with $CaCl_2$ and ethanol 9-10%. The use of tablets with potassium metabisulphite (MTB) also provides reduction of natural loss. The lower percentage of natural loss were obtained with the use of MTB (1g in film package), however in this variant skin browning was noticed (Table 6).

Table 6: Comparative results of grape stored at various variants of variety Taifi Rosoviy after 5 months of storage at 0C.

Variants	Rot, %	Natural loss, %	Standard, %
Control	12,0	9,0	79
6% $CaCl_2$	4,0	5,0	91
25% Ethanol	4,5	5,5	90
MTB 2g in air	9,0	6,0	85
MTB 1g in film	9,0	4,0	87

Increasing ethanol concentration used for the dip of grape to 35% ethanol showed better result than 25% ethanol. However, 50% ethanol caused more decay development and berry browning (Table 7).

Table 7: The influence of various pretreatments on trade quality of grape Taifi Rosoviy after 5 months of storage at 0C. Control = fumigation by sulphuric anhydride - SO_2

Variants	Standard grape %	Mass loss %	Rots %	Browning, %
Control (SO_2)	67,4	9,0	18,0	5,6
25% ethanol	83,3	8,7	6,0	2,0
35% ethanol	85,2	9,0	4,7	1,1
50% ethanol	79,2	8,8	9,0	3,0

Dipping table grapes in ethanol has been shown as a technology that can eliminate grape decay caused by *Botrytis cinerea* during storage (Lichter et al., 2002). This technique is sufficient for prevention of decay in the vast majority of circumstances for a storage period of one month or more. However, ethanol can eliminate external sources of infection but is not expected to handle internal latent infection. To overcome this obstacle, we develop in Israel a complementary modified atmosphere (MA) technique which is based on accumulation of CO_2 from the respiration of grapes.

In Israel, grapes of the variety Superior were dipped with ethanol (40%) and were packaged in plastic boxes with polyethylene liner with 0.2% perforation (0.2%PE) or with Xtend® (XFA) liners which are capable of accumulating CO_2 but have high permeability to water. These 2 liners created (MA) condition. Field pre-treatment included a biocontrol agent, which was applied 4 days before harvest and was packaged in both liners. After 6 wks at 0C both untreated and biocontrol-treated grapes at 0.2% PE suffered extensive decay. Only very few berries showed initial signs of decay in ethanol-treated berries or in XFA packages. After further incubation at 20°C for 3 days, decay in those treatments accumulated to an average of 35 and 75% of the berries, respectively (Fig. 3). In contrast, ethanol-treated berries which were packaged in XFA suffered only 8% of decay. Final CO_2 level in the XFA packages was 12.5%. Positive commercial control consisted of grapes packaged with 0.2%PE in the presence of slow release SO_2 pads (1 gr sodium metabisulfite per kg fruit) which maintained the berries free of decay. However, these pads caused extensive damage to the grape in XFA.

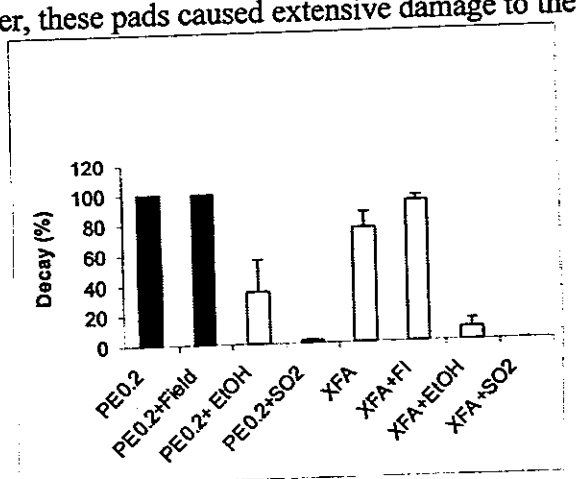
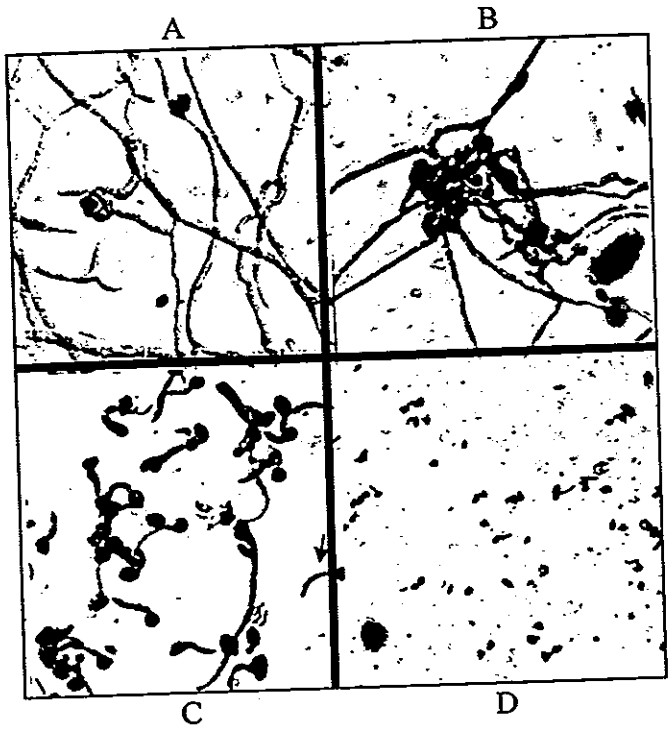


Fig. 3: Percent decayed berries in various liners (PE or XFA) after 5 weeks at 0C and 3 days at 20C of 'Superior' table grapes treated by EtOH, biocontrol (FI) and SO_2.

In Israel we study the possibility that AA which is formed from ethanol as a result of one enzymatic step by the enzyme alcohol dehydrogenase (ADH) is in part responsible for the control of the decay development.

Conducting *in vitro* studies show that application of AA vapors to the major grape fungi *Rhizopus stolonifer* and *Botrytis cinerea* prevent the growth of the fungi. The inhibition of the growth was expressed in every stages of the fungi development: germination of the spores, elongation of the germ tube and sporulation. In *Botrytis cinerea* a concentration of 0.4-2% AA inhibits the elongation of the germ tube and a higher concentration of 4% AA completely prevent germination of the spores (Picture 2). Application of 4% AA vapor was also significant in preventing sporulation of *Rhizopus stolonifer*.

Picture 2: Effect of various concentrations of acetaldehyde (AA) vapors on reduction in germination of the fungus *Botrytis cinerea* after 40 hours at 20C *in vitro*. Fungi colored with cotton blue 0.1%. A. Control B. 0.4% AA C. 2% AA D. 4% AA.



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C. Scientific Impact of Collaboration:

During the first year the collaboration was conducted only via email. Because the political situation in Israel Dr. Uryupina, the co-investigator in Kazakhstan did not find someone that agreed to come for training in Israel. However, now there is a candidate that will come for one year to Israel. Moreover, Dr. Uryupina intends to come to visit Israel soon. This visit will increase and strengthen the scientific connection between the two labs.

D. Description of Project Impact:

In both countries, Israel and Kazakhstan we are working on finding natural fruit coatings that will maintain the fruit quality for longer period. 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 improve fruit aroma and taste.

The treatment of grape clusters with ethanol dips was found to be effective in both countries on different cultivars of grapes. Whereas both ethanol dipping and MA packaging have the potential to prevent decay in table grapes, the combined technology has a superior potential which can offer quality assurance standards demanded by commerce. Further delineation is required to establish thresholds for decay prevention versus organoleptic quality of the berries. This technology can be adopted as a commercial treatment to replace the SO_2 treatments which are practiced today.

E. Strengthening of Developing Country Institutions

The collaboration between the two countries will lead to a flow of information about the new technologies in both countries. For example, Israel can advise Kazakhstan on how to make the new organic wax that is based on beeswax and expand it to other commodities besides apple. Examination of some of the bioactive components prepared in Kazakhstan, in Israel, will establish their efficacy. Application of ethanol dip to grape can be improved by the use of modified atmosphere packing. The research team in Israel can advise the Kazakhstan team how to apply ethanol in semi commercial setup.

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 material.
We are planning next year to concentrate on delay of ripening by blocking of the ethylene action, using a new gas compound 1-methylcyclopropane (1-MCP) which is ethylene action inhibitor.
To develop an ethanol dip system to grape clusters that can be done on larger scale and in combination with modified atmosphere.

Section II

A. Managerial Issues:

The first advanced payment of $ 7500 that was sent to Kazakhstan did not get to them because it was sent by regular air mail. The procedure which includes; cancellation of the first check and issue of a new check caused a big delay in starting the project. The first advanced payment arrived to Kazakhstan only in August 2002 , although the formal start of the project was June 2001.
For avoiding such problems in the future, the next checks should be sent to Kazakhstan not by regular air mail, but by UPS.

B. Budget:

Up to now each country got only the first advanced payment of $7500. For each country there is a sum of around $25,000, which is needed to accomplish the project.

C. Special Concerns

Because of the political situation in Israel, the research associate that was suppose to come to Israel from Kazakhstan to study our technology and to work with us in Israel, postponed her plans to come. Now we hope that Ms. Makhotina Alla Dastambekova who is intending to come for training in the lab in Israel will arrive in Israel in the near future. We already sent her a letter of invitation and hope it will be easy to get a visa for her from the Israeli Embassy in Kazakhstan.

D. Collaboration

Dr. Edna Pesis and Dr. Uryupina Tamara are in almost weekly connection via the email and fax. Dr. Uryupina sent Dr. Pesis a full report concerning the work in the first year via the email. We discussed together the results via the email. Dr. Uryupina Tamara is intending to come this coming year for a two week visit to our lab in Israel. In this visit we will be able to discuss together the progress of the project.

E. Request for American Embassy actions:

a. To pay a reimbursement payment for both countries a total of $15,000 so we can buy the instruments and chemical we need for the project.

b. The start of the project was postponed because of the significant delay in getting the advanced payment in both countries especially in Kazakhstan. We would like to ask to extend the project to June 2005 instead of June 2004, without addition of any extra money.