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A Method for the Cold
Storage of Grapes
in Lebanon

Constantin A. Rebeiz

PUBLICATION N° 7 (Série Technique) FEVRIER 1967

INSTITUT DE RECHERCHES AGRONOMIQUES
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CONSTANTIN A. REBEIZ, Ph. D.

CONTENTS

	PAGE
I — INTRODUCTION	1
II — SYMPTOMS OF GRAPE DETERIORATION DURING STORAGE	1
III — BASIC PRINCIPLES OF GRAPE STORAGE	3
IV — SUGGESTIONS FOR GRAPE STORAGE IN LEBANON	12
V — SUMMARY	14
VI — BIBLIOGRAPHY	16

MAGON. — Écrivain carthaginois qui a vécu vers 140 av. J.-C. Il écrivit en 28 volumes un Traité sur l'Agriculture et la Médecine Vétérinaire qui fut traduit en latin par ordre du Sénat.

Extrait de *L'Histoire de l'Agriculture Ancienne*
par A. ABOU NASSER, Beyrouth 1960.

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CONSTANTIN A. REBEIZ, Ph. D.*

I — INTRODUCTION

The table grape industry is an important agricultural activity in the Bekaa valley. However, serious marketing problems are bound to arise in the near future. Indeed local markets are already flooded by the october - november harvest. This in turn brings a severe drop in market prices. One possible remedy resides in controlling the flow of this commodity to market by stretching its marketing period over most of the year.

The purpose of this publication is twofold : to familiarize the lebanese extension scientists with the basic principles of grape storage, and to report on 9 years of personal experience in packing grapes for cold storage in Lebanon.

II — SYMPTOMS OF GRAPE DETERIORATION DURING STORAGE

Successful storage aims at preserving the grapes in an appealing marketable condition after extended periods of sto-

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rage. A perishable commodity like grapes renders this goal rather difficult. Grapes are easily subject to aging, decay, desiccation, and browning of the stems, with subsequent deterioration in market quality. Therefore, a review of the most important symptoms of quality deterioration during storage will achieve a better understanding of the principles of grape storage.

1. *Decay*: — The fungi that commonly cause decay in stored grapes are *Botrytis cinerea*, *Cladosporium*, *Penicillium* and *Aspergillus* spp. (19). These species seem predominant also in Lebanon (9).

Decay caused by *B. cinerea*, known in the trade as slipskin, is soft and brown (6). In its early stages, it is localized in the tissues just below the epidermis. A slight pressure applied to the berry causes the epidermis to separate from the underlying mesocarp tissue. In the more advanced stages of decay, the whole berry becomes brown, soft, and watery. A grey growth of mycelia and conidia may cover the surface of the infected berries. Under cold storage conditions, table grapes are highly susceptible to decay by *B. cinerea* (6).

Decay caused by *Cladosporium* spp is black and fairly firm. Under cold storage conditions, growth of this fungus is slow. Consequently decayed areas are usually localized and rarely extend over the whole berry, as does *Botrytis* decay (6).

At times, black or dark decayed areas, found mostly at the capstem attachment to the berry, are observed in Lebanon. The capstem is easily removed from the berry as if both ventral and dorsal vascular bundles are attacked. These symptoms are most noticeable on overmature grapes, left too long on the vines after cold rainy weather. At present the causal agent of these symptoms is under investigation by our plant pathology department.

2. *Desiccation* : — Stored grapes tend to lose water rapidly because of their relatively large surface-to-volume ratio, especially that of the stem. The first noticeable effect accompanying

water loss is drying and browning of stems and pedicels. This effect becomes evident with a loss of only 1 to 2% (1). When the loss reaches 3 to 5% the fruit softens, loses its turgidity and becomes mushy.

Successful storage is therefore achieved when decay is checked and water loss and browning prevented, without impairing the taste of the grapes. Accordingly, the next few sections will deal with the principles of decay and water loss control in stored grapes. Simultaneously the practical applications of the above principles will be considered.

III — BASIC PRINCIPLES OF GRAPE STORAGE

PRINCIPLES OF DECAY AND WATER LOSS CONTROL.

— *Storage at low temperature* : The necessity of low temperature storage arises from the general influence of temperature on reaction rates of physiological, chemical and physical processes. In general, the increase in the rate constant of any process, for a 10-degree change in temperature is denoted by the symbol Q_{10} . If one sets the rate constant of an imaginary reaction equal to unity at 0°C, then if $Q_{10} = 2.5$, the variation of the rate constant with temperature will be found to be:

Temperature	0°C	10°C	20°C	30°C	40°C
Rate constant	1	2.5	6.25	15.6	39

Uncatalyzed chemical reactions have a Q_{10} of 2 or 3 while in most enzymatic reactions the Q_{10} falls between 1.4 and 2.0. On the other hand the Q_{10} of physical processes ranges from 1 to 2. Now stored grapes, like any other living tissue, have a postharvest metabolic activity and undergo senescence during extended storage. Decay is the result of the activities of the

1. The Q_{10} of any process, physical, chemical or physiological, is defined as the number of times the rate of the process increases with a 10°C rise in temperature (11).

various microorganisms. On the other hand, water loss is mostly a physical phenomenon of water vapor diffusion. Therefore, the beneficial effects of low temperature storage are derived from a slowing down of physiological, chemical and physical processes, which in this case lead to aging, decay, and water loss.

Low temperature storage seems well suited for grapes. It causes no ill effect on texture, taste, or any other market quality. In practice grapes are stored between -1 and 0°C. (7). At -1°C no danger of freezing is encountered. The high sugar content and osmotic potential of the cell sap depress the freezing point of the berries below the above temperature.

— *Decay Control* : Decay control in cold storage is accomplished chemically by "surface sterilization" of the grapes with sulfur dioxide. Since 1925, this chemical remains the only effective means of decay control in grapes (1, 10, 17, 18, 20).

Sulfur dioxide (SO_2) is a colorless gas, 2.2 times as heavy as air. It has the pungent odor of fumes produced by burning sulfur. Its solubility in water amounts to 10^5 ppm, at room temperature. As a water solution, it is known as sulfurous acid ($\text{SO}_3 \text{H}_2$) At ordinary atmospheric pressure the gas liquifies at -10°C (20).

Chemically sulfur dioxide bleaches organic colors ; it combines with the chromagens to form colorless compounds. These compounds are easily broken up upon oxidation, and the color restored. Sulfur dioxide reacts readily with certain organic substances found in the grape, including the sugars, to form fairly stable compounds. This combination constitutes a major part of the bound SO_2 in the treated grapes. The uncombined portion is termed free SO_2 . It is this form of SO_2 which is a very active preservative (20).

Sulfur dioxide is an insecticide, disinfectant and a food preservative. Its mechanism of action on the intermediary metabolism of decay - causing microorganisms and host is not well investigated. On the whole, SO_2 appears to act as a potent,

wide spectrum enzyme inhibitor. It slows down the metabolic activity of both host (20) and decay - microorganisms.

In storage, decay results primarily from "incipient infections". These occur in the vineyard, but are not advanced far enough to be detected during the packing operation (5). During storage, these infections develop further and spread by contact from infected to sound berries. Sulfur dioxide "surface sterilizes" the fruits, that is, it kills fungus spores or mycelia on the surface of the berries. It has little effect on the fungus mycelium that has already invaded the tissue of the berry (6). In other words the fungus becomes independent of the outside environment of the fruit after incipient infection has taken place. There, arises the need of frequent contacts between the berries and SO_2 . In this manner, any mycelium that might reach the surface from the deep - seated pericarpic tissues is regularly controlled. Eradication of fungi from the fruit tissues, though possible, leads to serious damage to these tissues (14).

There is considerable variation in the amount of SO_2 injurious to different varieties of grapes, as well as lots of grapes within the variety (14, 15). The severity of the injury is usually proportional to the amount of sulfur dioxide absorbed by the tissue (15).

In cases of severe injury, colored grape varieties are bleached. In red grapes, the bleaching usually starts at the capstem attachment and progresses toward the other end of the berry. In addition to bleaching, the grapes assume a lifeless dull appearance (15). In blue grape varieties, the change in color is from blue to reddish blue. In white varieties, yellowing and dulling to an ashy pale yellow color takes place. Upon exposure to room temperature, browning of the fruit occurs in a day or two (15). It indicates cell injury and loss of organization within the cells. Injuries to the appearance of the grape are often accompanied by objectionable changes in taste.

In mild cases of SO_2 injury, a condition known as wetness develops (13). Moisture is smeared over the surface of many

of the berries during handling. When it dries, a sticky texture or varnish like appearance is imparted to them. In addition to wetness, decolorized sunken areas 1 to 5 mm in diameter impart a bleached appearance to the berries (13). Both phenomena are due to an excessive uptake of SO₂ by the fruit. Sulfur dioxide diffuses through lenticels and through injuries on the berry. At high concentrations, it causes loss of cellular semipermeability, localized damage and bleaching. The juice released by the injured cells diffuses out through these same avenues and results in depressed areas on the berry. The dried exudate imparts the sticky texture and shiny appearance to the fruit (13).

At adequate concentrations, SO₂ imparts an attractive greenish-straw yellow color to the stems. Without sulfur dioxide they would turn black. The intimate mechanism of this process is not well investigated. It might be due to the inhibition of chlorophyllase activity by SO₂ and a much slower rate of chlorophyll degradation. In addition, SO₂ might react with the stem pigments to form more stable complexes.

In practice, the aim of sulfur dioxide treatment is twofold: to bring the berries in contact with enough SO₂ to kill surface contaminants without injury to the fruit, and to prevent recontamination. This aim is achieved differently in various countries.

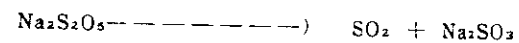
In the United States (1, 7, 10) grapes are fumigated with SO₂ for 20 minutes, immediately after the fruit is packed. Concentrations of 0.5 to 1% by volume of the unoccupied air space among the berries are used. If the fruit is subsequently stored, the treatment is repeated every 7 to 10 days as long as the fruit is held in storage. In that case, the fruit is fumigated with 0.1 to 0.25% SO₂ for 30 to 60 minutes. The fundamental principles of the fumigation process were reported by Jacob (8) in 1929. A more critical study of the factors affecting the movement and disappearance of the SO₂ in the fumigation area was published by Cant (2). Finally an up-to-date summary of the fumigation procedure could be found in Ashrae (1).

In other countries, the method of fumigation for decay control has not proven successful. In South Africa, Du plessis as reported by Cant (2) found that the quantities of SO₂ necessary to control mold made the fruit almost inedible. In Australia, De castella as reported by Cant (2) also mentioned similar findings. In both of these countries as well as in Lebanon (16), Sodium (or Potassium) metabisulphite² mixed with granulated cork is used with success for mold control. The metabisulphite provides a continuous supply of SO₂ during the storage period. Therefore, it surface-sterilizes the berries continuously. The granulated cork acts as a carrier for the uniform distribution of the metabisulphite in the grape box. It also cushions and insulates the berries from one another. In this manner, the berries are isolated from each other by cork granules which gradually release SO₂. Moreover, in case of localized infection the cork packing proved to be a very efficient barrier against the spread of infection to the rest of the box.

— *Water Loss Control* : Water loss takes place from the tissues of the berry to the outside through the numerous lenticels. This water loss is governed by well-known physical laws. The process occurs most probably in 2 steps : first, water evaporates from the cells whose wet surfaces are in intimate contact with the lenticular intercellular spaces ; second, water vapor diffuses through the intercellular spaces to the external air. The water vapor that diffuses out is replenished by evaporation from the wet surfaces lining the intercellular spaces, and so on. It is pertinent to mention that both filling tissue and phellogen of the lenticels have numerous intercellular spaces (3).

The passage of water from the liquid to the vapor state in the intercellular spaces of the lenticular tissue maintains a very high relative humidity in these spaces. The maintenance

2. Sodium metabisulphite : Na₂ S₂ O₅ is unstable and gradually decomposes to yield sulfur dioxide and sodium bisulfite according to the following reaction :



of this condition is an expression of the normal activities of the fruit tissue. The loss of this high humidity to the surroundings is concurrent with shrinkage and loss of turgidity of the fruit.

The passage of water vapor from the intercellular spaces to the outside obeys the general principles of gas diffusion. Any attempt at minimizing the water loss from stored grapes invariably amounts to a decrease of the rate of diffusion of water vapor from the intercellular spaces to the external environment. This rate, like that of any gas, depends on the density, temperature, diffusion pressure gradient³, and concentration of the medium through which diffusion occurs (11). Under commercial storage conditions, temperature and the water vapor diffusion pressure gradient remain the main controllable variables.

The rate of water vapor diffusion decreases at lower temperatures. Actual measurements of the Q_{10} of diffusion generally yield values between 1.2 and 1.3 (11). Besides at lower temperatures, air is saturated with water vapor more readily.

The rate of water vapor diffusion is depressed as its diffusion pressure gradient is lowered. The latter is most efficiently decreased by raising the water vapor diffusion pressure in the external environment of the fruit. Around 0°C, this is usually accomplished, by increasing the relative humidity in the outside atmosphere of the grapes. Ideally a relative humidity in the air as high as that of the intercellular spaces, at the temperature of storage, would bring the net water vapor outward diffusion to a standstill. This is hardly feasible and not advisable under commercial storage. Indeed maintaining a relative humidity of 90% is often a problem especially at the beginning of the storage season. At that time the rooms are being filled with dry lugs. Each 14 Kg lug will absorb from 150 to

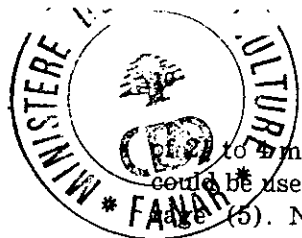
3. Diffusion pressure gradient : is equivalent to the difference in the diffusion pressures between the delivering and receiving ends of the diffusion system divided by the length of the distance between (11).

300 g. of water over a period of a month. Unless moisture is supplied to the room, this water must come from the fruit (1). Besides a very high relative humidity around the grapes might encourage the spread of decay in spite of the SO₂ treatment (18).

In the United States the relative humidity in commercial grape storage units is theoretically held at 85% to 95%. However, due to the difficulties of maintaining a high relative humidity at -0.5°C, the berries and stems are subject to desiccation in some cold storage houses (18). To check excessive water vapor loss, Uota in 1957 suggested the use of perforated plastic liners in the box (18). The degree of relative humidity inside the box and the extent of water loss depended on the amount of perforations per box. In this manner a microenvironment of optimum relative humidity could be established in every box. In Lebanon, perforated polyethylene liners were successfully used in 1958 to decrease water loss from stored grapes (16). That same year, the optimum number of perforations per box was determined.

— *Grape Quality and Cold Storage* : The use of grapes of good quality cannot be overemphasized. Indeed the treatment will not transform poor grapes into good ones (8). In the following discussion, the word storage quality will be used at length. By storage quality is meant the keeping quality of the grapes : that is, their resistance to decay during storage. It is meant also their market quality, that is the outside appearance and taste (desiccation, browning, SO₂ injury, off-taste) of the decay - free fruit.

— *Keeping Quality and Incipient Infection* : It was stressed earlier that decay control in cold storage amounts to a prevention of the spread of infection from affected to healthy berries. This initial infection of the berries cannot be detected by outside symptoms during the packing operation and is termed incipient infection. Harvey devised a simple test to determine the percent incipient infection in harvested grapes (4, 5). Moreover, he found a high correlation between the degree of incipient infection and the percent decay in grapes after storage



to 4 months. He concluded that laboratory determinations could be used to forecast the decay likely to develop during storage (5). Needless to stress that grapes of good keeping quality are of low incipient infection. Therefore, the understanding of the factors affecting the incipient infection of grapes becomes important.

Nelson studied the factors influencing the infection of table grapes by *Botrytis cinerea* (12). He found that the grapes were infected by *B. cinerea* through the uninjured skin. In other words lenticels, insect punctures and microscopic injuries were not essential courts of infection. The grapes became infected from conidia only when the berries were kept wet after inoculation. The length of the moisture period necessary to produce infection was a function of temperature. For example, under conditions of diurnal fluctuations in temperature and with a minimum temperature of 12°C, infection of mature Tokay grapes required a moisture period of 12 to 24 hours. With a minimum temperature of 2°C, 18 to 36 hours were required (12). Harvey confirmed these results (5). He reported that more decay from incipient infection occurred in lots of grapes harvested after rainy periods than in those harvested before the rain. It also appeared that decay caused by *B. cinerea* is much more related to rainfall than is decay caused by *Cladosporium herbarum*, *Alternaria* and *Stemphylium*, species (5).

Harvey in 1955 succeeded in reducing decay in stored grapes by preharvest applications of fungicides (6). The fungicides prevented field infections of the grapes and greatly reduced the losses from decay in cold storage. Captan (N-trichloromethyl-thio-4-cyclo-hexene-1,2 dicarboximide) applied as a dust was most promising. Better control of decay was obtained when dust applications were started early during the growing season. At that stage, the berries were small and free from one another in the cluster, thus allowing a good coverage. Complete coverage is difficult after the berries have matured and formed a tight cluster.

Harvey also noted that the percent incipient infection is

higher in late harvested grapes than earlier in the season (5). Although the early harvested grapes were in storage 7 weeks longer than the late fruits, they had much less decay upon removal from storage than fruits harvested at the end of the season. He concluded : "These results provide a conclusive answer to the question whether grapes destined for the late market hold better on the vine or in storage". In 1956, the same author confirmed his 1955 results (7). He suggested that late harvested grapes should not be stored for long periods but marketed soon after harvest.

Our personal observations for the last 9 years are in complete agreement with the results of Harvey and Nelson. Indeed consistently, better keeping quality was achieved with early - harvested, fungicide - treated fruits than untreated late - harvested ones.

— *Keeping Quality and Maturity* : Grape maturity is defined as that stage of physiological development when the fruit appears pleasing to the eye and can be eaten with satisfaction (1). Grapes destined for cold storage should not be left too long on the vine after acceptable flavor and color have developed. Overripeness predisposes the fruit to 2 serious post harvest disorders : weakening of the stem attachment and progressively greater susceptibility to the invasion of decay microorganisms (1).

In 1951, Nelson demonstrated that the sugar content of non-infected berries was significantly lower than that of the healthy tissue of infected berries in the same cluster (12). He concluded that berries high in sugar were apparently more susceptible to *Botrytis* infection. A positive correlation between wastage and maturity of grapes was also demonstrated by Rattery and Du plessis as reported by Harvey (7).

On the other hand, high acidity in the fruit seems to have a bearing on its keeping quality. It appears that high acid grapes absorb SO₂ faster (8, 15, 18). Besides, Gruess as reported by Jacob (8) demonstrated that as the hydrogen ion concentration of the fruit rises the proportion of free to fixed SO₂

increases. Jacob concludes that due to the toxicity of free SO₂ decay in high acid grapes should be easier to control than in low acid ones (8).

— *Storage Quality and Handling* : Injury of the fruit during the harvesting and packing processes lower both the keeping and market quality of the grapes.

Wounds on mature berries are not so important as avenues of entrance for the fungus (12). Nevertheless they are a substantial source of water and nutrients for the germinating spores and the spread of infection (12).

Several authors reported that grapes which are broken bruised or just weak in general constitution absorb SO₂ more rapidly than physically sound grapes (7, 8, 13). On the other hand the severity of SO₂ injury is proportional to the amount of SO₂ absorbed by the grape tissue (7, 13, 15). Therefore, handling injuries might become a serious source of market quality depreciation due to the increased probability of SO₂ injury and its accompanying effects.

— *Storage Quality and Variety* : Varietal considerations affect storage quality in so far as they influence sugar content at maturity, susceptibility to infection, physical properties of the berries and time of maturity. For example, juicy, thin-skinned, soft-textured varieties, are easily injured during handling. Besides they appear more prone to SO₂ injury than grapes with thick skins (8).

IV — SUGGESTIONS FOR GRAPE STORAGE IN LEBANON

The following method was worked out by the author in October 1958 (16) and since used for storing grapes in Lebanon for periods up to 6 months.

— *Procedure and Results* : The grapes are harvested dry. Injured and decayed berries are removed.

The boxes 16 x 30 x 50 cm. are lined with locally available polyethylene sheets. The polyethylene liner for one box is perforated with 96 holes 0.8 cm in diameter. These perforations are made to correspond with the upper and lower edges of the side panels of the lug box. In this manner the openings are not blocked by the sides or bottoms of the boxes. Twenty four holes are distributed on the upper and lower edges of each of the two side panels.

Sodium metabisulphite G P R⁴ grade or similar, is thoroughly mixed with granulated cork type 3/7 at the rate of 35.0 g per 3kg of cork for the red variety and 32 g for the white varieties.

A layer 1 cm thick of the metabisulphite-loaded granulated cork is placed in the bottom of the lined box. Then the box is neatly packed with the cleaned bunches of grape. The above lug would usually hold from 10.5 to 11 Kg of grapes, depending on berry size.

After the lined box is packed with grapes, it is filled with the metabisulphite-loaded cork until the surface berries are covered by a 1 cm thick layer of cork. This amounts to a quantity of about 1.5 Kg of cork per box. The loose sides of the liner are overlapped, fastened with scotch tape and the box cover nailed down.

The same day the packing operation is performed, the boxes should be placed in cold storage at -1 to 0°C and 85% relative humidity.

The above method proved very satisfactory for the cold storage of the red *Halwani* variety and less so for the white varieties. *Halwani* grapes of low incipient infection and of the right stage of maturity remain in a good marketable condition after 5 to 6 months of storage. On the other hand, equally good *Beitamouni* grapes should not be left in storage for more

4. G. P. R. designates a general purpose reagent complying with pharmacopia standards.

than 3 to 4 months. The *Sourivariety* if very carefully handled, would stand storage for about 2½ to 3 month. All attempts to store *Oubeidi* grapes failed due to the extreme juiciness and fragility of the berries.

DISCUSSION : — It was pointed out previously that early - harvested fungicide - treated grapes consistently outperformed untreated late - harvested ones.

However, the early harvest of the red *Halwani* variety is not always possible in the Bekaa valley. In most cases, the red color fails to develop fully at that time. Hence, *in vivo* and *in vitro* studies of the factors affecting the biosynthesis of the red anthocyanin pigment in this variety might be useful. It might help us control the development of that color at the right moment⁵. On the other hand, the application of fungicides during the growing season would be very useful. Actually, sulfur treatments for powdery mildew control might be combined with the applications of a compatible fungicide such as captan without additional labor costs. It is also most recommended to compute the index of correlation between incipient infection and decay in grapes stored under our conditions. This might give rise to a routine forecast of decay in lebanese storage grapes. A plant pathology laboratory like Tel Amara's is perfectly well-equipped to initiate such a program.

Although the above packing method has been proven over the last 9 years, we strongly recommend immediate research for the improvement of this method. Besides, the adaptation of SO₂ fumigation methods to our grape varieties would be extremely useful. By nature, a fumigation process is cheaper, easier and more fit for large scale commercial enterprises.

V — SUMMARY

The basic principles of grape storage were reviewed. A field method of packing grapes for cold storage purposes is

5. Research on this problem is already underway in the Department of Biological Sciences.

described. It makes use of Na-metabisulphite, granulated cork, perforated polyethylene liners and low temperature storage for decay and water loss control.

RÉSUMÉ

Les principes fondamentaux de préservation des raisins sont revus.

Une méthode d'emballage pour la préservation des raisins en chambre froide est décrite. Cette méthode utilise le métabisulfite de sodium, du liège granulé, des films en polyéthylène perforés et la réfrigération. Elle permet de contrôler l'infection et la dessiccation du fruit durant la période de préservation à froid.

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