EVALUATION OF POST-HARVEST TRENDS OF RESPIRATION RATES AND SOFTENING OF APPLES AND TOMATOES

J. E. BOEKE

634.11:631.563:581.12
635.64:631.563:581.12

EVALUATION OF POST-HARVEST TRENDS OF RESPIRATION RATES AND SOFTENING OF APPLES AND TOMATOES

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS DR. IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP DINSDAG 10 DECEMBER 1968 TE 16.00 UUR

DOOR

J. E. BOEKE, biol. drs.

H. VEENMAN & ZONEN N.V. – WAGENINGEN

STELLINGEN

I

Fysiologisch onderzoek van afzonderlijke specimina van vruchten of groenten is niet anders dan een verfijning van de gebruikelijke massale sortering op kwaliteit.

Dit proefschrift

II

Een vergelijkende studie van de bewaarfysiologie van gekoelde plantaardige producten vereist experimentele technieken die aanzienlijk minder dan 1% meetfout opleveren.

Dit proefschrift

III

Een monster vruchten of groenten dat meerdere exemplaren omvat is ongeschikt voor fundamenteel onderzoek naar het effect van luchtverversing.

CLAYPOOL, L. L. e.a. (1955): Proc. Am. Soc. Hort. Sci. **66**, 125–134.

IV

Inzake de stijfheid, hardheid of zachtheid van levende vruchten of groenten geven aperiodische of zeer laag frequente vervormingen meer informatie dan hoger frequente mechanische trillingen.

Dit proefschrift
Zie tevens: BOEKE, J. E. (1963): Landb.k. Tijdschr. **75**, 601–617.

V

De term 'percentage compression' is misleidend indien toegepast op tomaten of appels.

Dit proefschrift
SHAFSAK, S. A. en G. W. WINSOR (1964): J. hort. sci. **39**, 284–297.

VI

Amperometische titratie van een gegeven aanvoer van CO_2 is meer doelmatig dan de in dit proefschrift beschreven regelbare aanvoer van een absorptievloeistof van onbekende titer.

VII

Impedantie-metingen leveren bruikbare informatie inzake de bewaarfysiologie van vruchten en groenten.

BEAN, R. C. e.a. (1961): Yearbook Calif. Avocado Soc. **44**, 75–78.
BAIN, J. M. en F. V. MERCER (1964): Austral. J. Biol. Sci. **17**, 78–85.

VIII

De impedantie van een zaadkorrel is een geschikte maat voor de 'vigour'.

HEYDECKER, W. (1968); Preprint 54, 15th Int. Seed Testing Congress.
KOOSTRA, P. T. en J. F. HARRINGTON (1968): Preprint 15, ibid.

J. E. BOEKE

Wageningen, 10 december 1968

IX

De gebruikelijke wijze van inweken van zaden kan de uitslag van een 'viability test' ongunstig beïnvloeden.

LARSON, L. A. (1968): Plant Physiol. **43**, 255–259.

X

Voor het benaderen van een bepaald aantal korrels zaaizaad per maat-eenheid zijn volume-eenheden meer geschikt dan gewichts-eenheden.

BOEKE, J. E. en W. KUIPER (1966): Meded. Rijksproefst. Zaadcontr. **17**, 53–58.

XI

Ingeval een in duplo uitgevoerd zuiverheidsonderzoek van zaaizaden een ontoelaatbaar verschil tussen de twee uitkomsten oplevert, moet volgens voorschrift (I.S.T.A. International Rules, 1966) een tweede onderzoek in duplo worden uitgevoerd en zo nodig een derde en een vierde. Deze werkwijze is zinloos.

BOEKE, J. E. (1968): Preprint **61**, 15th Internat. Seed Testing Congress.

VOORWOORD

De aanvang van dit werk dateert van 1949, toen wijlen Ir. A. K. ZWEEDE, de toenmalige directeur van het Instituut voor Onderzoek op het Gebied van de Verwerking van Fruit en Groenten, mij opdroeg het lopende 'bewaar-onderzoek' uit te breiden met 'ademhalings-onderzoek'. Dat ademhalingsonderzoek ontwikkelde zich in een richting die niet geheel strookte met de doelstellingen van het Sprenger Instituut. Dat ik desondanks de gelegenheid heb gehad mijn eigen opvattingen te ontwikkelen tot dit proefschrift, stemt tot grote dankbaarheid.

Prof. Dr. E. C. WASSINK heeft mij ten zeerste aan zich verplicht door zijn bereidheid op te treden als promotor – op een gebied dat grotendeels buiten zijn eigen terrein van onderzoek lag – en mij te doen profiteren van zijn opbouwende critiek, welke de stoot gaf tot de ontwikkeling van mijn hydraulische hardheidsmeters.

In de vele jaren die zijn verlopen sinds het beëindigen van mijn studie te Leiden hebben ook talrijke anderen bijgedragen aan het tot stand komen van dit proefschrift. Naar alle dezen gaat mijn dank uit; ik wil echter slechts enkelen noemen.

Ik hoop dat JAKOB APELAND, Vollebekk, Noorwegen, destijds gast-medewerker op het Sprenger Instituut, het Nederlands nog voldoende machtig is om nu herinnerd te worden aan een bijzonder prettige samenwerking. Gaarne had ik hem laten delen in meer succes dan wij hadden met onze pogingen om het zachtworden van vruchten af te lezen uit resonans-spectra.

Ir. A. R. P. JANSE, Afdeling Landbouwscheikunde van de Landbouwhogeschool, heeft er mede toe bijgedragen dat die pogingen ten slotte werden opgegeven.

Drs. A. M. K. VAN BEEK en vooral ook Ir. G. BOREL, van de Stichting Technische en Fysische Dienst voor de Landbouw, vond ik steeds weer bereid tot het ontwikkelen van speciale apparatuur en het geven van deugdelijk technisch advies op velerlei terrein.

Het merendeel van mijn experimentele gegevens werd op verdienstelijke wijze verzameld door de heer T. HONKOOP; zijn toegewijde en nauwgezette assistentie leidde meermalen tot verrassende vondsten.

Ook anderen die tot het personeel van het Sprenger Instituut behoren of hebben behoord, met name mej. H. W. STORK en de heren Ir. Q. P. VAN DER MEER, F. COURBOIS en J. LANGERAK, hebben in belangrijke mate er toe bijgedragen dat nieuwe technieken werden getoetst en toegepast.

Mijn velerlei ontwerpen van glazen apparatuur werden verwerkelijkt dank zij het vakmanschap van de heer H. J. DE ROOY, glasblazer te Wageningen.

Dat de Senaat der Landbouwhogeschool zich bereid verklaarde mij, doctorandus biologiae, de graad van doctor in de landbouwwetenschappen toe te kennen, is mij een eer en een vreugde.

CONTENTS

1. INTRODUCTION

The market value of fruits and vegetables has a tendency to increase during storage. This tendency may reverse at the appearance of symptoms of a storage disease. Hence, economic losses are prevented when the storage is discontinued before the outbreak of a disease.

Elsewhere (BOEKE, 1961), the assumption was made that the appearance of the typical symptoms of a storage disease would be preceded by a deviation from the 'normal' trend of one or more physiological functions of the stored produce. If such a deviation could be detected immediately, appropriate measures could be taken, both to prevent economic loss and to obtain a better insight into the nature of a particular storage disease.

Normal trends may be defined as those resulting in *optimum market quality*. Some aspects of optimum market quality are open to quantitative evaluation; the same applies to the trends leading to these aspects.

Deviations from normal trends may occur in response to changes in the environment of the test objects. Other deviations can be considered *spontaneous* and are presumed to mark the onset of some kind of physiological disorder, e.g. a storage disease.

The phenomenology of spontaneous deviations must, of necessity, be studied under controlled conditions. Uncontrollable factors occuring in specimens stored together should be avoided, at least in the first stage of a quantitative study. Therefore, test objects should be single specimens, each enclosed separately in a container that provides the predetermined storage conditions.

Another reason for studying specimens that are isolated is that a spontaneous change of CO_2 production rate cannot be expected to happen simultaneously in different specimens. A one per cent increase occurring in one specimen in a composite sample of ten will most probably be overlooked since it causes only a 0.1 % increase in the combined production.

As a deviation may occur at any moment during the usual storage period, trends in individual specimens should be followed for weeks or months at a stretch without being interrupted or altered as a consequence of the observation. The type of research intended calls for techniques enabling observation from a distance, i.e. from outside the experimental place of storage, and allowing the test specimens to function the way they would under undisturbed storage conditions.

This excludes chemical and biochemical investigations which require disintegration of the test object. Studies requiring the test object to be temporarily transferred to a measuring device would not suit either as they might affect a respiration trend because of

- thermal stimulus,
- water vapour condensation, either on the outside of the cuticula or on the inside (GAC, 1955),
- disturbance of a wax layer,
- bruising.

Also visual inspection, necessitating illumination of the object, would interfere with trends that depend on constant darkness (WOLF, 1955, VAN DER MEER and WASSINK, 1962). Nevertheless, studies in storage physiology cannot do without visual inspection, probably.

Observation from a distance although excluding several sources of information leaves some ways open for investigation, for instance *gas exchange rate*. Among these the consumption of oxygen as well as the production of carbon dioxide, water vapour and volatile organic substances have provided useful information and continue to be of interest.

In addition *bioelectric measurements* have attracted interest (LUYET, 1932; ULRICH, 1946; CURTIS, 1950; SCHWAN, 1957; DE PLATER and GREENHAM, 1959; LABRIQUE, 1960, 1961 a and b; BOEKE, 1961). Changes of impedance were found by BAIN and MERCER (1964) in the surface of Williams pear, during ripening after cold storage; 50% decrease in three days was reported.

Among a few other items suggested by BOEKE (1961), a continuous record of *the intercellular gas composition* should provide a valuable counterpart of the data on respiratory activity of test specimens, according to ULRICH (1952).

Measuring intercellular gas composition as well as electric impedance requires the use of probes to be inserted into the tissue. As will be shown in fig. 20, III insertion of a probe having the dimensions of a hypodermic needle may cause the gas exchange of an apple to change only temporarily.

We also found no more than a very thin layer of affected (brownish) tissue around non-sterilized hypodermic needles that had been inserted into Cox' apples and left there several weeks at room temperature. The conclusion that chemically inert probes will do no harm when left inserted throughout the storage period of fruits, is confirmed by work of TROUT e.a. (1942), SMITH (1947), HULME (1952) and WILLIAMS and PATTERSON (1962).

It should be noted that each of the effects mentioned is non-specific; a change occurring in one of them may serve only as an indication that 'something is happening' in the test object. Coincidence of changes in two or more functions might give additional information. In the present study, special attention was paid to carbon dioxide production and softening in relation to external appearance – as will be described in chapters 4 and 5.

The effects to be studied should be measured while the objects are enclosed in suitable containers providing the predetermined storage conditions. A compromise has to be found between several requirements; many test objects will have to be transferred rapidly into the containers, an operator should be able to view the test objects from all sides at any time, and measurements must be possible throughout the storage period. We have attempted to realize these requirements in a conditioning machine, described in chapter 3. This unit, originally designed for respiration measurements, was called a 'respirotron'.

Conclusions as to the relation between a storage disease and the course of events preceding its outbreak, should be based on a large number of experimental data. In chapter 2, special attention is given to an efficient way of gathering, recording and compairing the data.

2. RECORDING OF TRENDS

The record of a particular trend in the storage life of a specimen may be a protocol, a table or a graph. Graphs are most convenient to handle when significant differences in various trends should be recognized without delay. This is necessary when conclusions are to be derived from the results of several series of measurements and when deviations from the 'normal trend' call for immediate action such as a biochemical analysis of the anomalous specimens.

Tables are indispensable when the significance of deviations between two sets of data is doubtful and should be evaluated by statistical analysis.

In physiological experiments series of data are most often provisionally recorded as tables and afterwards processed into graphs. Our data on CO_2 production rates and on gas flow rates were worked into graphs directly, saving man-hours and possible mistakes. Each trend was recorded as a series of dots, each dot showing a quantity at the time the reading was taken.

The practice of direct graphical recording implied that an operator had to carry out a series of actions many times in succession: connect a measuring instrument with a test object, take out the corresponding sheet of graph paper, take a reading from a scale, put a dot on the sheet, replace the sheet, and disconnect the instrument.

Precision and perseverance in this type of routine were greatly aided by the following factors:

- a span of at least ten centimeters between extreme readings on the measuring instrument;
- a few minutes to spare between two successive recordings;
- a time unit (day) of 1.5 mm on the abscissa of the graph paper, and
- one millimetre on the ordinate corresponding to one per cent difference between readings. The latter requires the use of a logarithmic ordinate with 20 cm unit length.

Recording had to be performed not only with optimal rapidity and accuracy but also should allow the graphs to be readily compared.

If 1.5 mm on the abscissa represents one day, and 1 mm on the ordinate represents 1% difference, a 200 days' record of the respiratory activity of a specimen would occupy a surface of 10×30 cm^2. A hundred graphs laid side by side would provide a general view of a hundred trends, but would be very impractical; an investigators eyes would have to travel either over a distance of thirty meters or over a surface of three square meters all covered with dots.

This discouraging prospect does not exist if the viewing is done with the aid of a technique apparently not applied in similar investigations elsewhere, to make dot diagrams on transparent plastic foil (0.06 mm cellulose acetate has been used, as well as 0.125 mm polystyrene) with a sheet of graph paper lying underneath. A number of such graphs, when stacked properly and viewed through would reveal significant deviations (discontinuities) that might otherwise escape attention. This is demonstrated in figs. 10 and 11. In fig. 11 the dots have been omitted, for easy comparison.

Whenever a sheet was put in position on top of the others and wiped with a dry cloth or with filter paper or even with the back of the hand, electrostatic adhesion prevented shifting during viewing of the graphs, but did not hinder regrouping of the sheets. For semi-permanent adhesion, when a pile of graphs was to be photographed, each sheet was sealed on top of the others by puncturing one or two of its corners with a hot soldering iron.

Cellulose acetate or polystyrene can be written on with Indian ink, after the film has been rubbed with dry cloth or filter paper. Although the ink adheres strongly, when dry, it can be wiped off easily with moistened filter paper. As an aid to rapid recording, the high price of the film is compensated by the saving in labour.

3. DESCRIPTION OF THE CONDITIONING MACHINE

3.1. Introduction

For the purpose of measuring CO_2-production rates, the object should be placed in an atmosphere of unchanging temperature and composition. Also, each test object should be visible from all sides at any time wanted.

Both conditions are met when a test unit consists of a thermostated, ventilated, transparent container enclosing a single specimen. The ventilation rate should be such that the CO_2-content of the atmosphere inside the container (and of the gas mixture released through its outlet) will not rise much above that of air, otherwise respiration rates are known to be reduced (Claypool et al. 1955). For lemons, Biale and Shepherd, as cited by Biale (1950), found 0.2% CO_2 to be an upper limit. It may be safe to assume that for exact measurements on apples, the CO_2-content should not surpass 0.1%. On the other hand, to simulate storage conditions, it should not be much lower than that, so that the respiration containers should not be flushed excessively. In work with single apples, each producing 0.5 to 1.0 mg CO_2/hour, an hourly supply of 1.0 to 1.5 litres of CO_2-free air would keep the CO_2-content in the containers somewhere between 0.015 and 0.05%.

For these slow respiration rates, the Pettenkofer method of CO_2-measurement is rather inadequate (Biale, 1950). Both the space required for the necessary parallel sets of Pettenkofer tubes, as well as the amount of work required to obtain the data (according to Claypool and Keefer, 1942, the output of one operator is 20 to 25 tests a day) would not allow the concurrent study of a reasonable number of samples.

The method of Claypool and Keefer is claimed to be much more rapid, enabling one person to take 40 readings in an hour. However, to express results as milligrams CO_2/hour, the readings need correction for barometric pressure. In the case of rapid and frequent barometer changes a source of errors is introduced that may add considerably to the 3.5% error claimed to be inherent in the method.

The same applies to respiration studies made with other types of instruments measuring CO_2-*percentages*, such as the katharometer (diaferometer) and the infrared absorption meter. An estimate supported by personal statements of users of these instruments in this country, shows the speed of consecutive measurements on individual apples to be about the same as with the Pettenkofer method. This is because the individual apple containers have to be connected to the instrument one after another. The low gas flow rates (about one liter/hour), the dead volume of the apparatus, and other factors would result in a relatively slow reaction of the instrument.

The ideal method should allow rapid reading of respiration rates (of apples) while sets of readings should be converted into graphs without previous correction for barometric pressure.

Ventilation of the test produce at *constant pressure* – by a method described below – was considered advantageous. Without that, any percentage of CO_2 read in respiratory gases would need a correction for barometric pressure. Also, a rising barometer would have a retarding effect on a flow of air leaving a container, and in this way simulate a decreased output of CO_2. A falling barometer would more than compensate for a previous output that had been retarded since it would cause some of the expanding intercellular gas – containing several per cent of CO_2 – to escape into the container atmosphere, increasing once more the output of CO_2. Also, the ventilation of the intercellular space caused by fluctuations in the atmospheric pressure would add an uncontrollable physiological factor to the response of the test object.

Considering these disadvantages the arrangement commonly used in respiration measurements with open circuit has been modified as described below.

3.2. Control of simulated storage conditions

3.2.1. *Constant pressure*

Fig. 1 shows[1] how the test objects were ventilated at a constant rate by means of an aspirator[2], a constant air flow resistance and a constant pressure difference between the inlet and the outlet of the ventilating system.[3]

The pressure difference was regulated by means of an overflow. The system did not exhaust into the open atmosphere, but via an outlet tube that was immersed in the same water column as the overflow, securing a constant pressure difference regardless of the water level. The absolute pressure in the system was kept constant by having the water level rise and fall to compensate for changes in the barometric pressure (figs. 2 and 3).

This is how in each apple container a pressure of about 1080 millibar was maintained constant to within 0.01 millibar. The high pressure enabled rapid detection of leaks, a valuable aid that more than compensates possible objections against the increase of the partial pressure of oxygen (108% of the normal value) to which the test fruits were exposed.

3.2.2. *Gas-tight connections*

More than in any other part of the ventilating circuits gas leaks occurred in the metal-to-glass connections of the apple containers (fig. 4). In the course of a storage period, at least one out of every ten metal-to-glass connections, however carefully made, would sooner or later show a leak (see section 3.2.3). When discovered by an apparent decrease either of the CO_2-supply or of the air-flow

[1] The legends of figs. 1–5 constitute a running description that is commented on in the text of pages 6, 12 and 13.

[2] The aspirators mentioned in this work were all of the vibrating diaphragm type; capacity at zero pressure difference 1.5 to 2.5 liters/min; maximum pressure 15 to 20 cm/Hg; suction 6 to 12 cm/Hg. Unfortunately the type used has been taken off the market.

[3] It was not considered necessary to have the air humidified before it entered the containers as shrivelling of test objects (Jonathan apples) did not occur at an alarming rate.

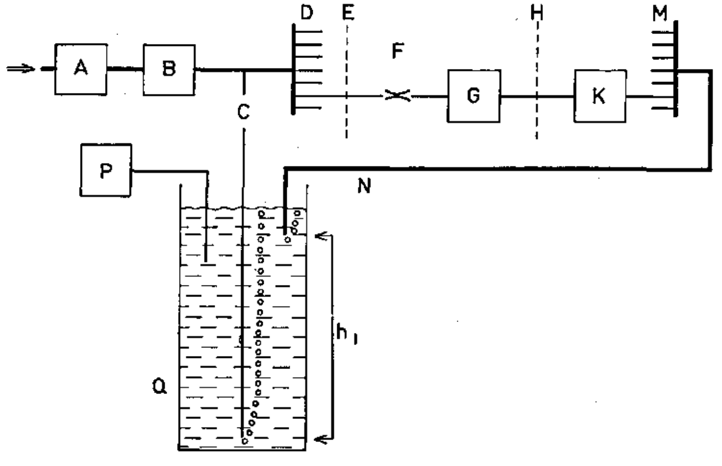


Fig. 1. General outline and flow diagram of the ventilating system. A flow of air, about 2 liters/min, leaves compressor A, is dried and made CO_2-free in trap B, partly escapes through overflow tube C and is divided in the manifold D. Each of the branched-off leads passes an air flow resistance F and a fruit respiration chamber G. The leads are reunited in manifold M and the air escapes through exhaust tube N which is immersed in water tank Q. In each of the containers G the air flow rate, the absolute pressure and the temperature are kept constant. Constant air flow rate is achieved with the aid of a constant pressure drop (h_1 = 1.2 m water column) along the capillary air flow resistance F (details in fig. 4) The pressure drop is negligible upstream from F as well as downstream from F. The absolute pressure is kept constant since fluctuations of the barometric pressure are compensated by movements of the water level in Q, commanded by the pressure regulating system P (details in figs. 2 and 3). The temperature is kept constant by thermostat E-H (details in fig. 5). At K apparatus for measuring air flow rate (details in fig. 8) or for measuring CO_2 production rate (details in figs. 6, 7 and 9) can be incorporated without disturbing the air flow through a container G.

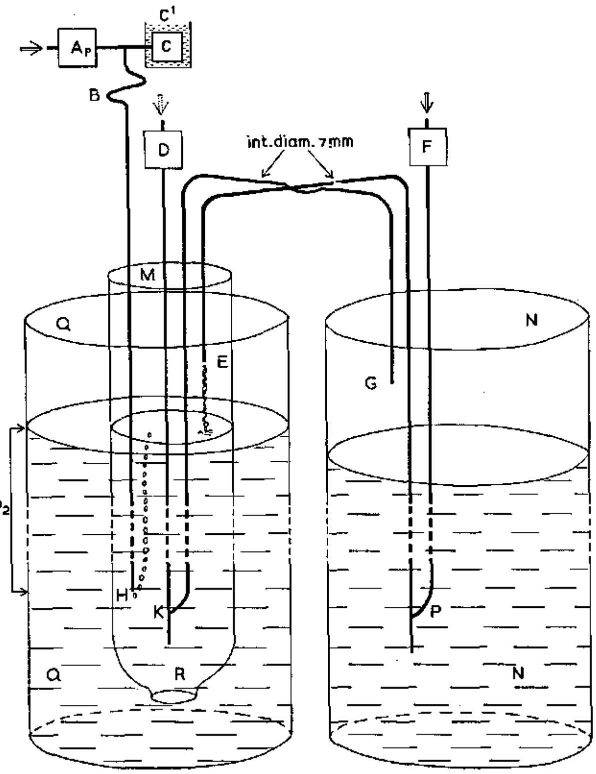


FIG. 2. Principle of pressure regulating system (details of fig. 1-P)
A small flow of air, about 1 liter/hour, leaves compressor A_p and escapes through exhaust tube H, the depth of immersion (h_2) of which can be regulated because B is flexible. The hydrostatic pressure h_2 is transmitted to manometer C (details in fig. 3) which by closing or breaking an electric circuit commands the air compressors D and F. The cylinder M, 2 meter long and 4 cm inside diameter, is fixed inside water tank Q (corresponds with Q in fig. 1); M encloses the exhaust tube H as well as the intake K of airlift pump system D-K-G- and the discharge E of the airlift pump system F-P-E; by means of the hole R, diameter 2 cm, the cylinders M and Q communicate. The water reservoir N has the same size as Q: height 2.4 meter, diameter 25 cm; water can either be taken from it via F-P-E or discharged into it via D-K-G. Pumping causes the water level in the cylinder M either to rise or to fall. The hydrostatic pressure transmitted to manometer C will change accordingly. If the total pressure (hydrostatic plus barometric) is below a predetermined value, D-K-G automatically stops pumping and F-P-E starts. The reverse occurs when the total pressure in manometer C exceeds the predetermined value. The antagonistic pumping systems cause about 20 cm^3 water to be transferred either from or into cylinder M every two or three seconds. This alternating action causes the water level in M (and in Q) to oscillate around a certain position that rises and falls in contrast to the barometric pressure. The amplitude of the oscillation is about 2 cm in M but less than 0.2 mm in Q because the restricted communication between the two cylinders acts as an attenuator and causes these rapid fluctuations to be flattened out. In a range between 970 and 1040 millibar, any change of the barometric pressure was found to be completely compensated, which allowed the tank Q to be used as a manostat as shown in fig. 1-C.

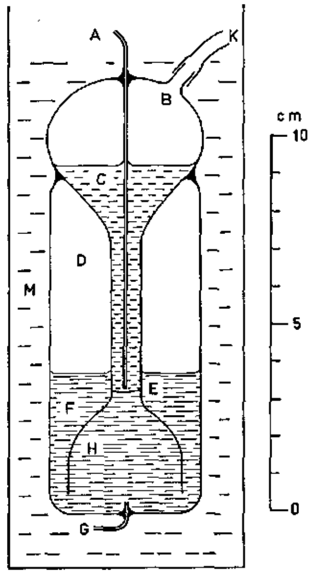


FIG. 3. Pressure regulator (detail of fig. 2-C).
This is essentially a closed U-tube dual-liquid manometer. Its closed end D and its open end B are co-axial. B is connected by means of flexible tube K to the pressuresensitive system (fig. 2-AH). The regulator contains mineral oil C floating on sulphuric acid H-F. Movements of the liquid interface E make and break the electric contact between electrodes A and G. The electrodes are connected to an electronic relay that commands the air-lift pump systems shown in fig. 2; the relay operating on a few microwatts prevents the formation of gas bubbles on the electrodes. The regulator is enclosed in a transparent water bath through which cooling water M from the respirotron circulates; it is placed in such a way that the interface E is easily visible. As parts B and D are wide, an incidentally large rise or fall of the liquid interface E will not allow an escape of gas from D or of mineral oil from B.

Preparation of the pressure-regulating system. The system must be adapted to the barometric conditions prevailing in the area where it is used. The water tank (fig. 2-Q) should not overflow at a low barometric pressure, nor should its level fall below the exhausts (fig. 1-N, fig. 2-H) in a high-pressure period. Therefore, the lowest and highest barometric pressures ever registered locally should be taken into account. If they are e.g. 970 and 1040 millibar respectively, the critical lower level of the water tank (fig. 2-Q) is 1040–970 = 70 cm below its brim. If the barometric pressure at the time the regulating system is being set to work is e.g. 1010 millibar, the tank should be filled with water up to 1010–970 = 40 cm below the top. The exhaust (fig. 2-H) should be fixed provisionally about 50 cm below the critical level, that is 70 + 50 = 120 cm below the top of the water tank.

The manometer is placed upright and filled with the appropriate volumes of acid and mineral oil – acid first. The mineral oil should be coloured, e.g. with a Sudan dye. To see whether the volumes of the two liquids are suitable, the air pressure in B is changed for instance with the aid of a hypodermic syringe connected to K till the liquid interface reaches the position shown in fig. 3, By then the positions of the other two liquid levels should also be as shown in fig. 3. If necessary a little acid and/or oil can be added to (or removed from) the manometer with the aid of an all-glass syringe connected to a piece of flexible plastic tubing of 20 cm length and 3 mm outside diameter.

As soon as the two liquids both have reached the right volume the manometer is connected to the pressure-sensitive system (fig. 2-A-H) and the water mantle is connected to the cooling system of the respirotron. When temperature equilibrium has been reached, the liquid interface is brought to the level shown in fig. 3 by either lowering or raising the exhaust (Fig. 2-H). If no appropriate position can be found over a vertical distance of more than a meter between the bottom and the critical level of the water tank. a little air should be added to or removed from D. For this purpose K has to be disconnected from the system (fig. 2-A-H). To remove some air from D, suction is applied to K. To add some air to D, the thin plastic tubing (that was previously used for transfering acid and/or mineral oil) is inserted down to the bottom of the manometer, and a little air is made to bubble through F.

As soon as the liquid interface is in the right position the pressure regulating system should be set to work. Additional corrections can be made by raising or lowering the exhaust (fig. 2-H), which should not be fixed above the critical level.

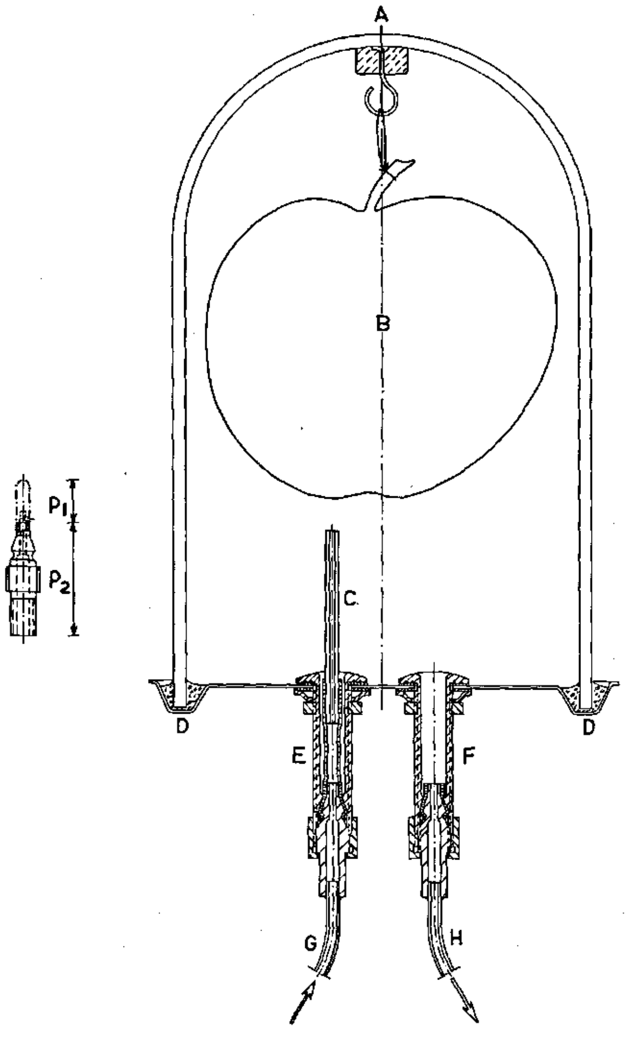
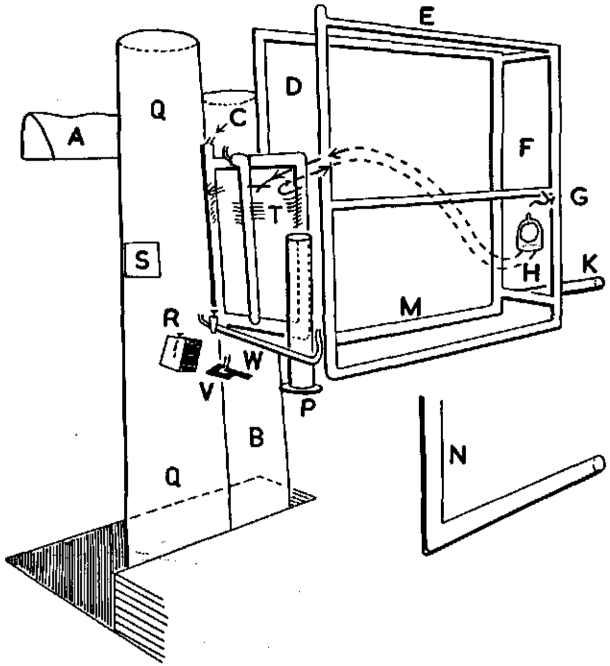


FIG. 4. Apple container
Apple B is suspended in a dome-shaped glass jar, by means of a loop of metal wire, a small hook and a piece of cork A that has been cemented inside the jar, with picein wax. The glass jar is cemented (with a mixture of picein wax and anhydrous lanolin) to a copper disk that is provided with a circular groove D, and with tubules E and F. The tubules hold the air inlet G and outlet H, the construction assuring leak-proof connection and easy removal (bicycle tire valves with the tip p_1 clipped off were found suitable). The inlet is connected to an air flow resistance C (a few centimeters length of thermometer capillary, 0.1 mm bore, that keeps the air circulation rate at about one liter/hour and that is easily replaced in case of failure). When the bell jar is to be connected to or disconnected from its metal bottom, G and H are removed, the ventilation rate is increased to one liter/min (by means of an auxiliary aspirator) and the circular groove D is heated with a micro-flame. The heat and the fumes generated during the heating are removed by the strong ventilation.



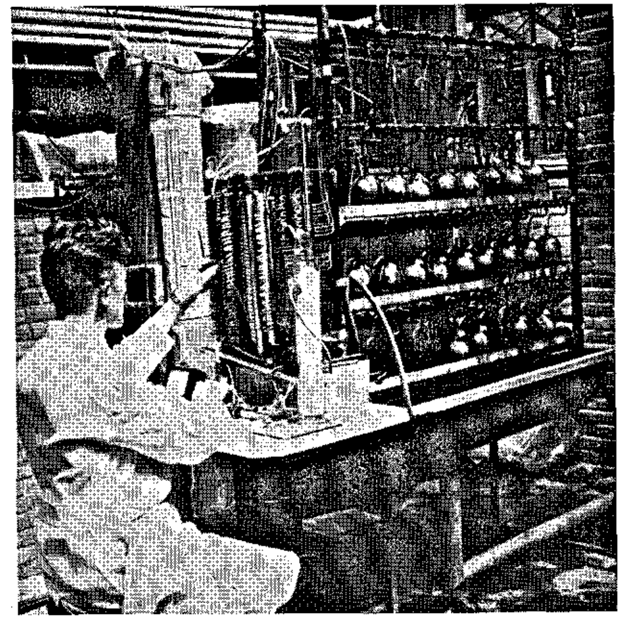


FIG. 5. *First version of the respirotron, cooled with spring water (10.8°C).* Cooling water is pumped, through K, into a frame of tubing D-E-F-M which serves as a support for platforms holding apple containers H, and also serves as a manifold bearing water taps G. Water runs over the apple containers H and down into drain N. Compressor A is covered with a small tent; after having passed a filter, the air flow from A is introduced at C into a rectangular frame of tubings that serves as a manifold (see fig. 1-D). A second manifold (see fig. 1-M) bears two sets of tubes T, both left and right. These tubes are provided with X-pieces, two limbs of which are closed during normal ventilation; they are opened when air flow rate or CO_2 production is measured. Q is the manostat cylinder (see fig. 2-Q). The cylinder B serves as a water reservoir (see fig. 2-N). The performance of three outlet tubes (see fig. 1-C and N, also fig. 2-H) can be supervised through window S.

On the table before the operator are an electric stopclock R, a micrometer screw clamp V (see fig. 9-E) and a slanting burette W (The latter served as an air flow meter, before the type shown in fig. 8 was used). Glass cylinder P contains a CO_2 absorption vessel of a type used previous to the one shown in fig. 6.

rate – which was checked once a week – a leaky container had to be removed from the respirotron, its metal bottom had to be heated with a flame, and the connection had to be remade.

The temporary change was found to affect the test fruit in the container, the result being a sudden rise of its CO_2-production, which would not regain its previous rate until several days later (fig. 20).

3.2.3. *Improved type of container*

Although the containers described above have been in use for four consecutive storage periods, they did not entirely suit the purpose. It takes 15 minutes to assemble one and this is too much for prompt transferral of several tens of specimens into the experimental conditions. Tests have been made with another type of container, consisting of two transparent plastic hemispheres (poly methyl metacrylate, diameter 10 cm, wall thickness 1 mm) provided with 2 cm flanges that are glued together with luting wax. Air inlets and outlets, the same as those depicted in fig. 4, are screwed in the plastic hemispheres. So far, these containers are satisfactory, both in speed of assembly and resulting gas tightness.

3.2.4. *Thermoregulation*

The containers were arranged in two rows on metal platforms, so that the test apples could be studied from all sides, if necessary with a dentist's mirror. Between inspections each container was suffused with water of constant temperature. A jet of water, about one liter/min, was directed onto each container from a soft copper tubule provided with a tap. A detergent (a quaternary ammonium compound added to the water) made the water run down from all sides of each container as an unbroken film, without causing excessive foaming. After having passed the containers, the water ran down into an insulated tank, from which it was pumped up again to the taps via a manifold.

A contact thermometer was positioned in the exhaust of the water pump; by means of an electronic relay it directed the compressor motor of a refrigerating unit the evaporator coils of which were in the water tank. The compressor made one start and stop about every half hour, keeping water temperature fluctuations within 0.1 °C. An aluminium foil tent lightly covered the apparatus, providing additional thermal insulation of the containers.

An earlier type of respirotron, without the refinements that were added later, is shown in fig. 5. A more recent type is covered with a protective case. In the latest version, the containers – of the kind described in section 3.2.3 – are suspended in an insulated tank and sprayed with the circulating water.

3.2.5. *Evaluation of CO_2 diffusion*

Each of the 72 test objects required about three meters of 2 mm bore tubing for connection with the two manifolds D and M (fig. 1). At first, copper and stainless steel capillaries were used and connections were soldered or made with short pieces of rubber tubing. Later, faulty parts were replaced by polyethene tubing, 2 mm bore, 4 mm outer diameter.

CO_2-diffusion through this tube proved negligible. This conclusion was based on gravimetric tests. In these tests, a one meter length of the tubing was flushed with CO-$_2$-free air, which then passed a series of sodium asbestos absorption tubes, while the tubing was surrounded with virtually pure CO_2, flowing through a glass mantle. No more than 0.65 mg CO_2/hour was found to diffuse along the given gradient of 100 to 0% CO_2. The gradients existing in our apple respiration studies may have induced both inward and outward diffusion, as inside the tubing 0.015 to 0.1% CO_2 would occur, and 0.03% outside of it. As calculated from the above data, diffusion rates in both directions were likely to be less than 0.001 mg CO_2/hour at all times, which is negligible in comparison to the apple production rates of 0.5 to 1.0 mg CO_2/hour.

Among the different types of determination that are of interest in the study of gas exchange of apples, the measurement of CO_2 production rates has been chosen. The method adopted is described in chapter 4.

4. MEASUREMENT OF CO_2 PRODUCTION RATES

4.1. METHODS

4.1.1. *Introduction*

The method used to measure CO_2 production rates was based on direct titration, as described by BLOM and EDELHAUSEN (1955). These authors determined CO_2 in air by titration with sodium methanolate. They led air from a compressor through two absorption vessels connected in series. Both vessels contained acetone (the CO_2 dissolving capacity of which, at room temperature, is about eight times that of water) with thymol blue added as a pH indicator. The pH of the mixture was raised to the thymol blue range (pH *tb*) with 0.01 N sodium methanolate in methanol (a sharper colour change than with methanol as a solvent was said to be obtained with a methanol-pyridine mixture, 1:4 (vol/vol)). To maintain pH *tb* during the passage of the air, the flow of the methanolate titrant into both absorption vessels was manually controlled at a rate corresponding to the flow of CO_2 absorbed. After equilibrium had been maintained for fifteen minutes, the volumes of air and titrant used were read from a gas flow meter and from two burettes respectively; the CO_2-content of the air sample was calculated from the titer of the methanolate solution, which was determined with the aid of benzoic acid.

The results reported warrant the conclusion that the mixture in the first absorber vessel, while being kept at pH *tb*, would absorb virtually all the CO_2 from air passing at a rate of 2 liters/hour.

4.1.2. *Modifications of the method of Blom and Edelhausen*

In order to measure CO_2 production rates of individual apples, the method of BLOM and EDELHAUSEN has been modified as follows.

4.1.2.1. Constant gas pressure

As described before, fluctuations in barometric pressure were automatically compensated in the ventilating system; the stream of gas through each apple container had a constant pressure P_a of for instance 1080 millibar. This pressure was maintained constant during analysis since the scrubbed gas escaped at a variable pressure P_b, being P_a minus the variable pressure drop in the absorber.

In the 1962/63 season, the pressure drop in the absorber was reduced to zero with the aid of an auxiliary aspirator as shown in fig. 6, and the somewhat awkward manipulation with P_b was no longer necessary.

4.1.2.2. Indicator

Thymolphthalein was used as an indicator; its shift between blue and colourless allows equilibrium to be observed better than with thymol blue.

4.1.2.3. Absorption vessel

The modified vessel (fig. 6) allowed the titrant to be administered continuously. It also allowed less than 2 ml of the liquid to circulate. This small volume can be expected to increase sensitivity at least six times as compared to the vessel described by BLOM and EDELHAUSEN, which held at least 11 ml. Although the

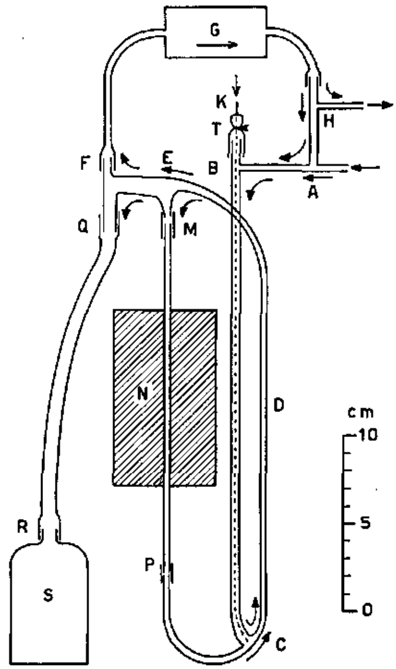


FIG. 6. Micro-scrubber for direct titration of CO_2 (fig. 3–K).
Parts B-C and P-C-D-E are sturdy borosilicate capillary tubing, 2 mm bore; thin -walled borosilicate tubing M-P, 2 mm bore, serves as a cuvette in the photoelectric attachment N (see fig. 7); connections M and P are gum rubber; Q-R is 6 mm bore vinyl tubing. G is an aspirator pump adjusted to a constant flow of 3 liters/hour that circulates through H-A-B-C-D-E-F. Co-axial with glass tube B-C is a capillary conduit K-C, 0.3 $\times$ 1.0 mm polyethylene tubing, led through a short piece of gum rubber vacuum tubing that is constricted at T to provide a gas-tight connection; titrant solution is forced through this capillary (see fig. 9-A). Gas and liquid are mixed at C creating an air-lift system in C-D-E and are separated again in the widened upper part E. The gas is led through F; the liquid returns via M-P, while any excess is drained through the flexible tube Q-R into the receiver flask S. By tilting the apparatus, the circulating volume of liquid is kept at a minimum, so that no air bubbles are carried down M-P and no liquid enters F. At A respiratory gas is introduced into the system, its flow rate is lower than the rate produced by the recycling pump G so that the respiratory gas is scrubbed in the airlift system C-D-E at least once before it escapes through H. The lowering of the pH of the titrant caused by absorption of CO_2, induces discoloration of the thymol phthalein in the titrant. This discoloration is observed with the aid of the photoelectric attachment N (see fig. 7). It is compensated manually, the operator controlling a flow of titrant through K-C as shown in fig. 9.

retention time of the bubbles in the air lift was less than half a second, absorption of CO_2 from a flow of air up to 2.5 liters/hour was evidently complete, as will be discussed below. The active volume of the vessel could not be further reduced without perceptibly impairing absorption; it was also found that the smaller volume of absorbent showed an increasing tendency to raise its pH when ventilated with CO_2-free air. This may be due to an increase in concentration because of evaporation of acetone, and/or to hydrolysis of the carbonate present caused by water taken up from the CO_2-free air, which was not dried before entering the absorber.

Because of the high surface/volume ratio of the apparatus, and the low normality of the circulating liquid, the use of non-alkali (borosilicate) glass tubing is essential.

4.1.2.4. Titrant

A 0.001 to 0.004 N solution of potassium methanolate in a methanol-acetone mixture, 1:4 (vol/vol), was used instead of sodium methanolate. In the latter mixture, CO_2 causes precipitation of sodium methyl carbonate (WENDLANDT and BRYANT, 1955) which necessitates frequent cleaning of the absorption vessel, and interferes with photoelectric colorimetry (see fig. 7). In addition, the indicator appears to be adsorbed at the precipitate. This was demonstrated by comparing methanolate solutions of potassium and of sodium – or lithium – both coloured with thymolphthalein, almost neutralized with CO_2, and kept in securely stoppered test tubes. The potassium mixture remained clear, and retained its blue colour several days; in the sodium and the lithium mixtures, after settling of the precipitate, the liquid was colourless within a few hours.

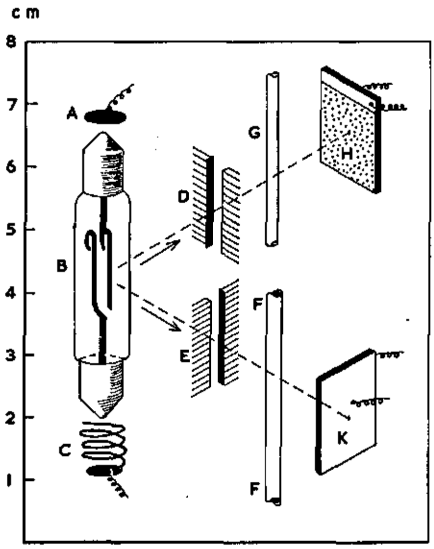
4.1.2.5. Determination of gas flow rates

The technique used is not specified by BLOM and EDELHAUSEN. We used a modification (fig. 8) of the soap film calibrator described by GOODERHAM (1944) which enables absolute values (volume/time relationships) of gas flow rates between 0.5 and 2.5 liters/hour to be determined at a rate of about thirthy readings per hour, with an error of about 1 %.

4.1.2.6. Determination of titrant flow rates

BLOM and EDELHAUSEN measured volume/time relationships directly; they read the level of the titrant in a burette at the beginning and at the end of exactly fifteen minutes of delivery. During that time they maintained a desired titrant flow rate, equivalent with a given supply of CO_2, by manipulating the burette stopcock – in other words by controlling the resistance to liquid flow between a batch of titrant and the absorber.

In our investigations a fixed capillary resistance (A in fig. 9 and K in fig. 6) was used, consisting of 40 cm of PORTEX polyethylene tubing, Pt 49, 0.35 mm bore, enclosed in a sheath of nylon tubing that served both as a protection and as a barrier against diffusion of CO_2 from the atmosphere. An intermediary short piece of narrow bore rubber tubing with a pinch clamp served as a stopcock. The flow rate was controlled by variation of the pressure difference between a batch of titrant and the absorber. To compensate a given rate of supply of CO_2 the pressure difference had to be manually adjusted to the



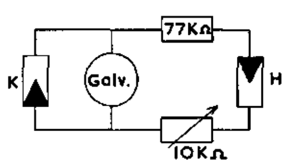


FIG. 7. Electrophotometer for CO_2-titration (exploded view of parts enclosed in holder.)
Light from neon gas discharge tube B (PHILIPS Z 11 T, maximum current 2 mA, clamped between electrodes A and C – with spring – and fed with stabilized current), passes slits D and E, cuvette tube F-F (corresponding to M-P in fig. 6) and compensator glass rod G. It activates indicator photocell K and compensator photocell H (selenium barrier layer photo-elements, 10 × 15 mm surface); infrared and colour filters are superfluous. Galvanometer circuit: the 10000 Ohm variable resistance is turned until deflection of the galvanometer (we used type A75, KIPP, Delft, Holland portable light spot galvanometer, internal resistance 450 Ohm, maximum sensitivity 7×10^{-6} Volt, 0.3×10^{-9} Amp.) is zero when the liquid circulating through F-F is colourless. A colour shift to pale blue induces full scale deflection of the galvanometer; the sensitivity is highest when the galvanometer deflection is mid-way. That position is maintained by manual control of the titrant flow as shown in fig. 9.

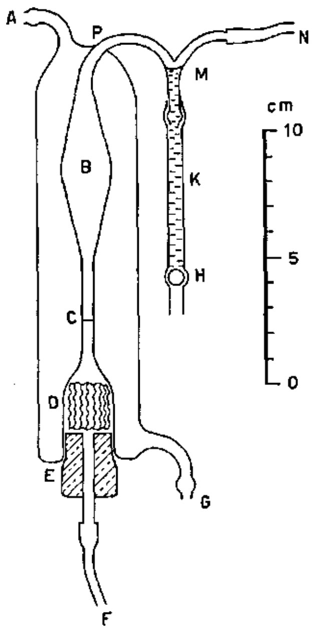


Fig. 8

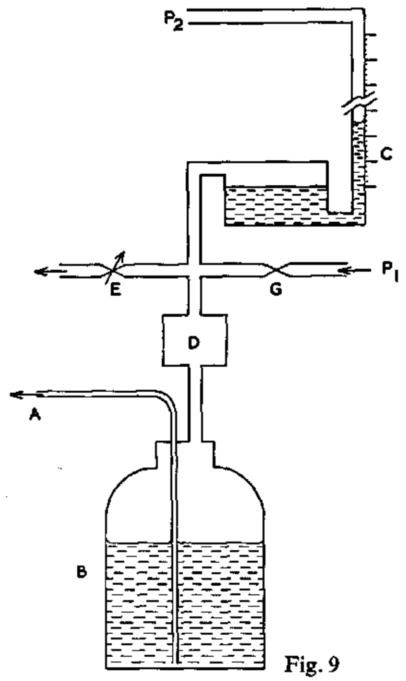


Fig. 9

FIG. 8. Micro gas flow meter (fig. 3-K).

Rubber tube K attached to Y-piece M (4 mm bore) is closed with glass bead H that is pushed upward until the level of a detergent solution (dishwasher detergent, diluted) in K is close to the parting of M. The flow of air of be measured enters at N with a standardized pressure and leaves at F with virtually the same pressure. Pinching K causes a soap film to be formed at M, which by force of the flow of air is pushed down B, past ring-mark C and on until it is taken up into a loosely rolled piece of filter paper D. The pipette volume B between parting M and ring-mark C, about 10 ml, is determined gravimetrically; the time allowing a soap film to travel from M to C is measured with an electric stopwatch, AEG Sekundenmesser, type 200 s, one revolution in ten seconds, totalizing 200 seconds. The inner surface of the pipette should be perfectly smooth – especially at the weld P – and free from sudden corners. Rubber stopper E is removed when filter paper D is renewed. Cooling water from the respirotron flows through glass mantle from G to A.

FIG. 9. Pneumatic control of titrant flow.

A small flow of air from P_1 (a constant source of 1200 millibar) is made to pass resistances G and E to the open atmosphere. G is a length (about 5 cm) of glass thermometer capillary, 0.1 mm bore. E is a variable resistance constructed as follows. A short length of glass fiber lined silicone rubber tubing is provided with a rubber sheath, gum rubber vacuum tubing, and clamped in a folded steel strip that is fastened in a micrometer; by turning the micrometer screw the tubing is clamped more or less tightly, providing a variable pressure drop. The variable pressure between G and E forces titrant from supply bottle B through plastic capillary A; the pressure exerted can be read from manometer C, connected to a source of constant pressure $P_2 = 1080$ mb.

The manometer is filled with kerosene (boiling range about 195°C) coloured with Sudan III. The titrant supply bottle B is protected with CO_2-trap D, filled with soda asbestos. Capillary A extends into the micro-scrubber (fig. 6 K-C).

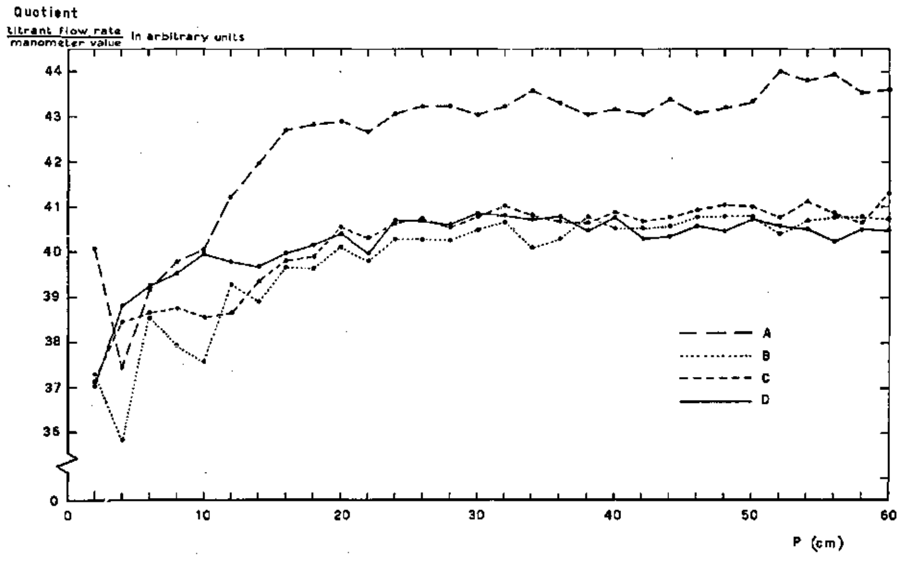


FIG. 10. Rate of flow of titrant as related to pressure read on manometer (see fig. 9-C). For the sake of differentiation the ordinate shows regression factors instead of flow rates, rectilinear regression appearing as a horizontal line. Below 20 cm pressure, a deviation from rectilinear regression occurs. Between 30 and 60 cm pressure flow rates increased 0.80 cc/min in A, and 0.73 cc/min in B, C and D. Series A, B and C have been taken all from the same batch of titrant, series D was from another batch, which was apparently equivalent. The higher regression coefficient of A cannot be explained. Series A was taken 5 days before B; series B was 2 days before C; series C was 6 days before D.

'equivalent manometer value'.

The relation between CO_2 supply rates and equivalent manometer values will be discussed in section 4.1.4. Titrant flow rates at a given set of manometer values were determined as follows. The titrant delivery (plastic capillary) tube (K in fig. 6) was disconnected from the absorber and connected to a receptacle, a 1 cm^3 microburette with closed stopcock. By means of a T-piece and an auxiliary tube, the receptacle was kept at pressure P_2 (see fig. 9) during delivery. The time needed for the transfer of 1 cm^3 of titrant served as a base for the calculation of the titrant flow rate at a certain manometer value.

The manometer scale was adjusted so that the zero manometer value corresponded exactly with zero flow rate of the titrant; to that end, a small air bubble was sucked into the plastic capillary K-C in fig. 6 and the pressure difference was adjusted until the bubble did not move in either direction (In our measurements of apple respiration, with the capillary tube connected to the absorber, the indicator bubble was frequently used for checking the zero point at the manometer scale).

The relation existing between manometer values and titrant flow rates was virtually rectilinear, provided the manometer values exceeded 20 cm kerosene column (see fig. 10). Comparing a, b, c and d in fig. 10 the relation appears rather inconsistent. This necessitated frequent checking against a known rate of CO_2 supply, a test gas, as will be discussed below. The apparent deviation from the rectilinear range at lower manometer values had to be considered whenever a series of readings had to be transformed, as will be discussed below. It required the titer of the absorbent to be made as low as feasible.

4.1.3. *Rate of testing*

An experienced operator could accomplish seventy tests in four hours. He would know approximately the settings of the air pressure control (E in fig. 9) corresponding to an expected manometer value, find the required pressure in about one minute, and maintain it another minute before reading the manometer.

4.1.4. *Relation between rate of supply of CO_2 and equivalent manometer value*

To establish this relation, different rates of CO_2-supply, both from apples and from samples of compressed air from a commercial steel cylinder, were measured gravimetrically, after drying with magnesium perchlorate and absorption in soda-asbestos (6 samples) or in soda lime (16 samples). Two corresponding equivalent manometer values were determined, one before and one after an absorption period of 24 hours, and their average value was taken.

Gas flow resistance was shown to be negligible in the granular absorbent. We used four absorption tubes, inner diameter 6 to 7 mm, alternately containing 10 grams magnesium perchlorate or 10 g soda asbestos or soda lime, 10 to 14 mesh. The resistance was negligible too during direct titration when an auxiliary aspirator was used as described before. The samples chosen covered the whole range encountered in previous measurements of apple respiration.

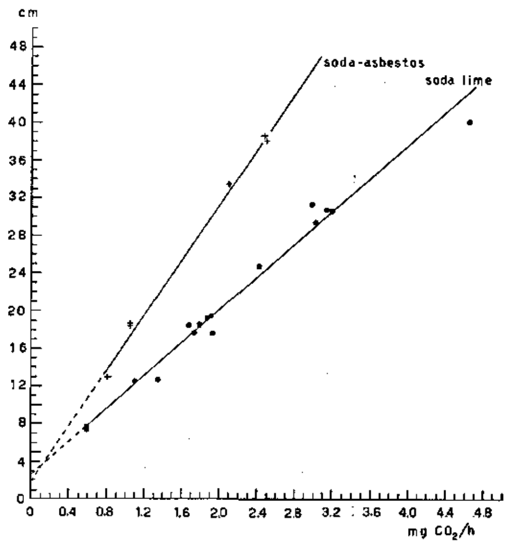
The relationship appears to be linear (fig. 11). The regression coefficient depends on the chemical and physical properties of the titrant; decrease of titer and increase of viscosity and density both increase the regression coefficient.

An equivalent manometer value applying to 'zero CO_2' can be found by extrapolation. It was expected to correspond with the 'zero titrant flow rate' discussed before. However, extrapolations based on gravimetric determinations of CO_2 or on test gas flow rates such as in fig. 12, produced positive manometer values of 2 cm and 5 cm respectively. We have been unable to explain this discrepancy.

On the other hand, the extrapolated manometer value corresponding with 'zero CO_2 supply' of apple respiratory gases coincides with zero titrant flow, as may be concluded from figs. 13 and 14. This coincidence permits linear transformations to be made.

In consequence, fluctuations, not due to physiological causes, can be corrected with the aid of (estimated) group transform coefficients as described in the notes with fig. 13. Concurrent trends of respiration rates of different apples can be compared by using the readings of one as a standard to the others (see (fig. 14).

FIG. 11. Relation between gravimetry and manometry. Each datum is the result of (i) gravimetry after 24 hours absorption in a granular absorbent and (ii) averaging the two equivalent manometer values (see fig. 9-C) read before and after the 24 hours absorption period.



Both types of transformation of graphically recorded data have been performed repeatedly, resulting in the trends to be discussed in section 4.2.

4.1.5. *Accuracy and precision*[1]

Five examples may illustrate the accuracy and precision of the method.

Fig. 11 shows a close linear relationship between manometer readings and gravimetry. That relationship cannot be evaluated with the aid of a correlation coefficient, because the data do not satisfy requirements of homogeneity. The relationship can be judged with KENDALL's rank correlation method, that is an evaluation can be made of the probability that the outcome of gravimetry does *not* depend on manometer readings (null hypothesis). The null hypothesis is not acceptable however, as its probability is between 5% and 2.5% with regard to the data on soda asbestos, and smaller than 0.5% with regard to the data on soda lime. The close correlation makes the absorption-gravimetric method an appropriate standard as discussed in section 4.1.6.

Daily routine measurements involved taking consecutive readings every 3 to 4 minutes of apples numbered 1 to 72. Immediately after number 72, the numbers 1 to 10 were measured a second time. The next day readings started with nos. 11 to 72 and continued with 1 to 20; the third day the sequence would be nos 21 to 72 and 1 to 30... etc. In that way duplicate readings were made with a time interval of about four hours. The difference (regardless of the + or — sign) between these duplicate readings was on the average 1.22% of their mean value in the 1959–60 season, 0.96% in 1960–61, and 0.97% in 1961–62.

In November 1961, the respiration rates of 41 apples (Jonathan and Boskoop)

[1] The author wishes to acknowledge the assistance of Mr. T. Honkoop who cooperated in obtaining the experimental data reported in this chapter.

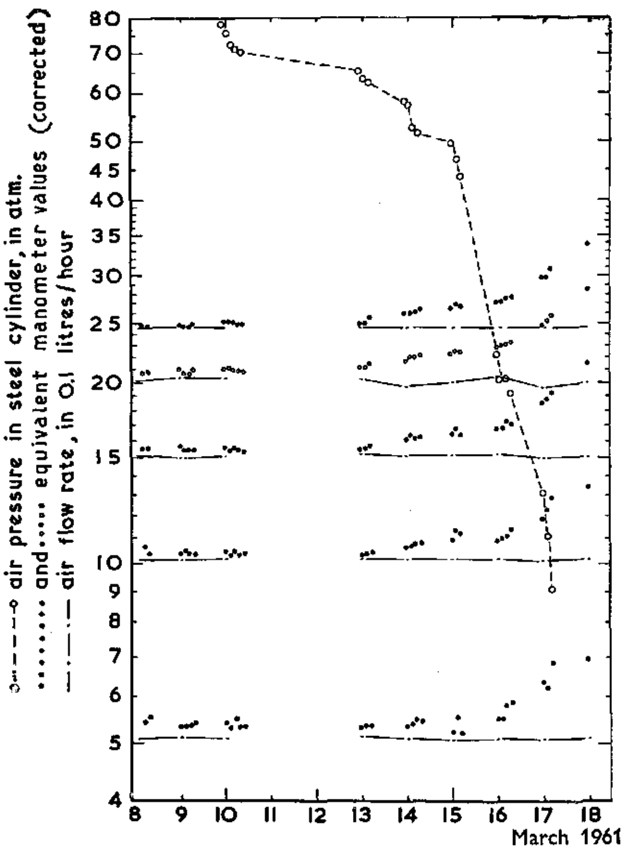


FIG. 12. Results of tests with compressed air from a steel cylinder that was gradually emptied. *Cylinder pressure* dropped from 150 atmospheres down to 9 atmospheres, as read on the manometer built in the reducing valve of the cylinder. The air was taken through five different flow resistances. The resulting five *flow rates* were measured daily. Also the *CO_2 content* of the five air streams was determined repeatedly, the „equivalent manometer values" showing concurrent increase in the course of time. At the lower cylinder pressures CO_2 apparently was released, which had been absorbed in or adsorbed at the inside of the steel cylinder.

FIG. 13. Trends of CO_2 production of five specimens (selected from 60 analogous ones) of Jonathan apple. Each fruit was ventilated individually. Flow rates in liters/hour read as follows:

(a) 0.965, (b) 1.023, (c) 1.054, (d) 1.440, and (e) 0.962.

I: equivalent manometer readings showing four sudden transitions, the occurrence of which coincides with and has presumably been caused by

B- on December 5 the titrant delivery system was revised;

C- on December 18 the titrant stock solution was renewed;

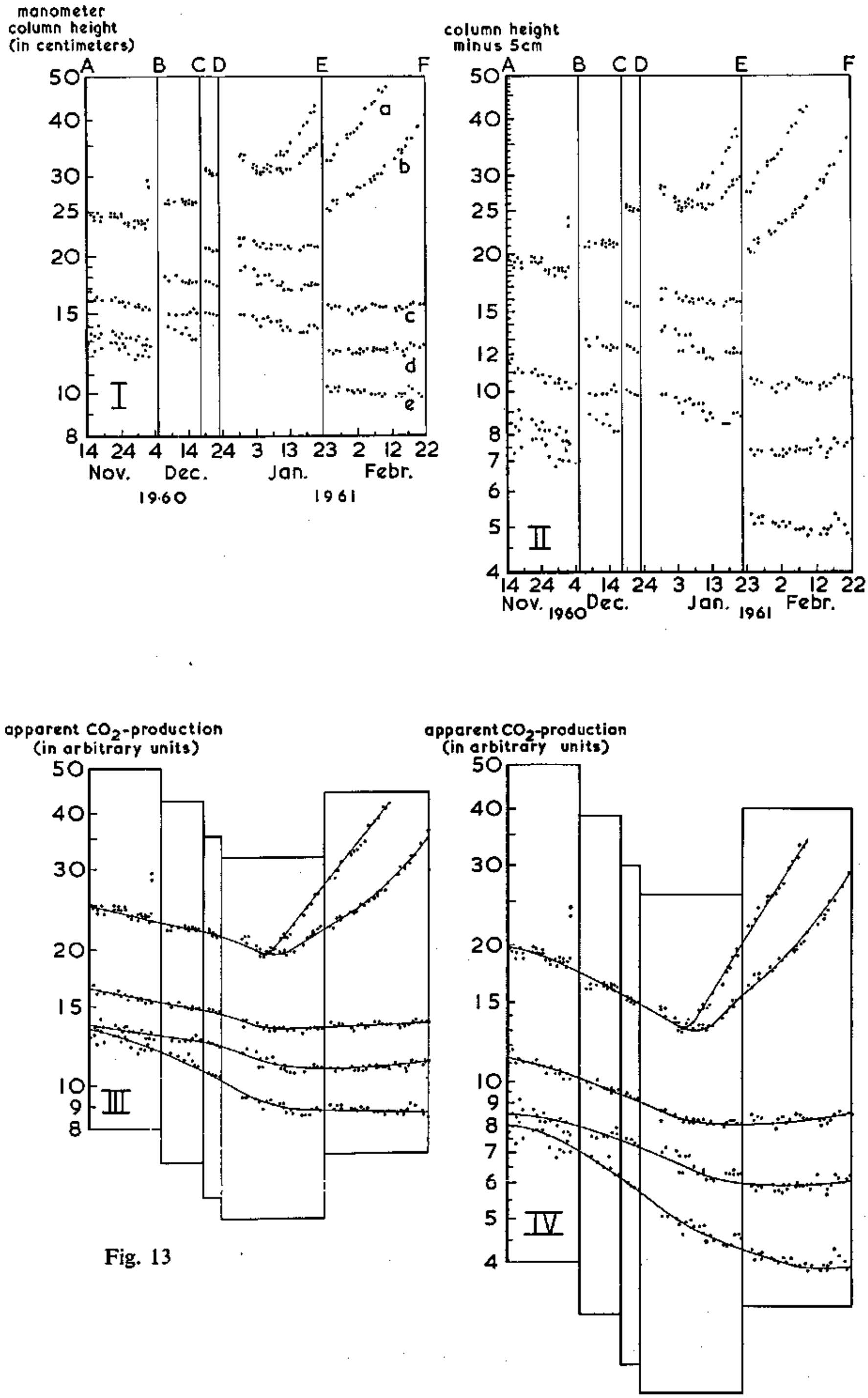
D- on December 23 the pressure regulating system was revised;

E- on January 22 the titrant stock solution was renewed.

II: registration of data differing from I in that each has been reduced by 5 cm.

III: sudden transitions in I were removed by group corrections.

IV: sudden transitions in II were removed by group corrections; the previous reductions of the data does not seem to improve the result of group corrections.



manometer column height (in centimeters)
column height minus 5cm
A B C D E F
a
b
c
d
e
I
II
III
IV
14 24 4 14 24 3 13 23 2 12 22
Nov. Dec. Jan. Febr.
1960
1961
apparent CO_2-production (in arbitrary units)

Fig. 13

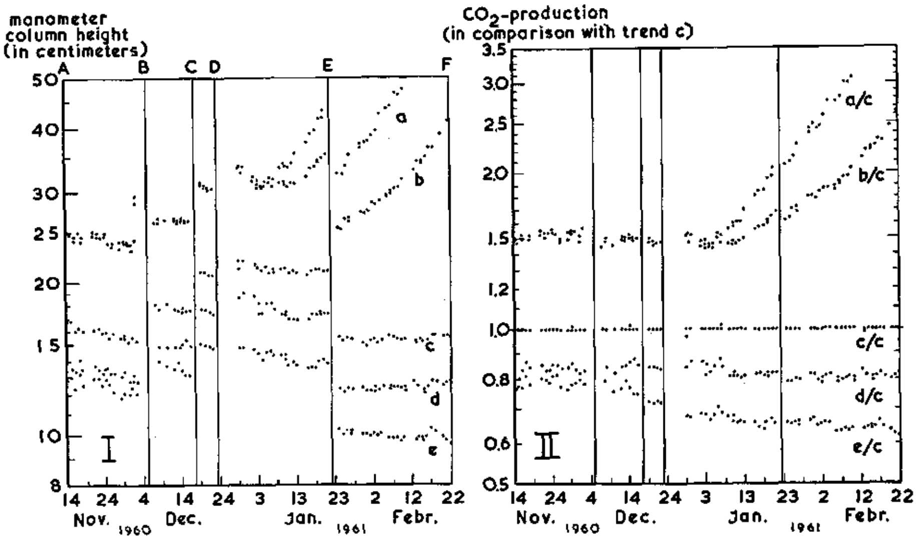


FIG. 14. Comparative trends of CO_2-production of five specimens of Jonathan apple, in relation to one of them.
I: non-processed data of five trends (same as fig. 13, I);
II: proportional values (graphically designed) of the data, those of c presumed to be 1.00.

steadily declined; but on the 14th when a new batch of titrant was put into use, all equivalent manometer values exceeded those of the previous day and necessitated a group correction as described in the notes with fig. 13. The ratio of corresponding data on November 14 and 13 was 1.196 ± 0.027. Consequently the variation coefficient of the correction factor is 2.3% (i.e. 0.027/1.196) which is not much larger than the variation of duplicate readings – between which four hours have elapsed – being about 1%, see above.

As shown in fig. 12 the data obtained from March 8 to 13, 1961, provided fourteen replicates of a virtually unchanging quantity. The standard deviation of the readings taken of the 0.5 liter/hour series is 1.4% of their mean value (the latter being reduced by 5.2 cm), and in the other series it is 0.8% (in 1.0 l/h), 0.5% (in 1.5 l/h), 0.7% (in 2.0 l/h) and 0.7% (in 2.5 l/h). The similarity of the variances in the latter four series, and the apparent linear relationship between gas flow rate and equivalent manometer value suggest that CO_2 is completely absorbed from samples up to 2.5 liters/hour.

As the standard deviation of equivalent manometer values is about 1%, the method described allows the 0.03% CO_2 content of air to be measured with an error of 3 parts per million.

The smallest volume of air measured has been 8 ml (passing in one minute at a gas flow rate of 0.5 liter/hour), containing 2.4 mm^3 CO_2. At that slowest rate the standard deviation was 1.4%; consequently, the limit ($P = 0.05$) of sensitivity may be considered to be $1.64 \times 0.014 \times 2.4\ mm^3 = 0.055\ mm^3 = 0.11 \times 10^{-3}$ mg CO_2.

4.1.6. *Standardization*

When trends of CO_2 production are compared, each trend can serve as a standard to the others. But an independent standard is needed for the evaluation of the absolute values of CO_2-production and of the CO_2-levels in the container atmospheres.

Test objects have been compared with standard flow rates of air taken from a commercial steel cylinder. The reference air samples were taken until the cylinder pressure, initially 150 atmospheres, had gone down to 80 atmospheres. This limit was set because the CO_2-level of the compressed air, when initially $a\%$ appears to rise to 1.4 $a\%$, as may be concluded from fig. 12. This could be due to a certain amount of CO_2 absorbed in (or adsorbed at) the rusty inside of the steel cylinder and released at the lower pressures.

A cylinder of 10 liters capacity enabled reference air samples such as given in fig. 12 to be taken daily for several weeks, before the cylinder pressure had become critical. An interim renewal of the reference air cylinder usually necessitated the application of a correction factor as shown in fig. 13.

With due regard to the limits set by the critical cylinder pressure, reference air samples are very useful as a check on possible drifts and other artifacts in respiration measurements. But reference air samples cannot provide a basis for the calculation of absolute values, such as a CO_2 production rate or a CO_2 level in a container. That is because the CO_2 percentage in a cylinder can be any value between 0.03 and 0.06%, as I verified gravimetrically. Absolute values are to be derived from gravimetric determinations, one or two of which should be included in the daily routine measurements. In our work gravimetric standardisation was done infrequently as our results were meant to be comparable only.

4.2. RESULTS AND DISCUSSION

4.2.1. *Deviations occurring in the pre-climacteric stage*

Apples, picked and stored as usual in commercial practice, produce CO_2 at a rate that changes during storage. A course as shown in fig. 15 has been repeatedly found (see ULRICH, 1952). The so-called climacteric rise is characterized by the date of its onset, the rate of CO_2 production at the start, its steepness (the percentual increase per unit time, in mid-phase), and its height (which may be double the rate at the start). The characteristics mentioned may be more or less obscured by secondary effects due to changes the fruit has undergone before, during and after picking.

Such unknown secondary effects may have produced the three successive groups of curves, I, II and III as shown in fig. 16. But in the first gathering the fruits had passed through similar environmental influences; still, the climacteric curves fall apart into two groups, with different steepness. From August 25 to 28, 1961, the relative increase in the one group (8 specimens) was 28.0 $\pm$ 6.1%, in the other group (11 specimens) 6.6 $\pm$ 3.5%. The difference is highly significant. The two lots did not show any significant dissimilarity in regard to the average fresh weight per fruit and the loss of weight during storage; also, the two lots showed a similar average rate of CO_2 production relative to the original fresh weight, between September 6 and 8 during a virtually level course after the climacteric.

The curves show that a significant difference between small lots of apples (8 and 11 specimens) is detectable within three days (August 25 to 28) in the preclimacteric stage, if the daily increment in CO_2-production surpasses the experimental error.

The magnitude of the daily increment depends on the nature of the fruit and on its temperature. In the preceding example the daily increment was abnormally high; if supposed to be exponential it was 2.2% in the one lot and 8.6% in the other. That is because of the inherent high metabolic activity of the James Grieve apple variety – which is not recommended for long-term storage therefore – and the high temperature which is several degrees above recommended temperatures for apple storage. The climacteric rise of a more slowly respiring apple, Bramley's Seedling, is reported (KIDD and WEST, 1945) to take 190 days at 2.5°C or 7 days at 22.5°C. If a 70% increase is assumed to be reached exponentially the daily increment would be 0.18% at 2.5°C, which is far below the increments 2.2% and 8.6% mentioned before. Apparently, under normal storage conditions a difference between two preclimacteric increments might *not* be noticed within three days unless the experimental error is further reduced.

4.2.2. *Post-climacteric trends*

4.2.2.1. The normal course

The above restriction also applies to the post-climacteric decrease of CO_2-production. As reported by KIDD & WEST (1945) and as shown partially in fig. 16, the post-climacteric course normally is a steady decline that may last weeks or months, until it is more or less abruptly modified. With Bramley's Seedling at 12°C, KIDD and WEST (1945) found a daily decrease of 0.56% in a typical case. In the samples shown in fig. 16 (James Grieve, at 10°C), it was 2.3% on an average; in 61 specimens of Jonathan apple, kept at 3°C in the 1959–60 season, I found 0.06% to 0.55% (mean 0.26%). Evidently, the preclimacteric rise as well as the postclimacteric decline of the late varieties, at the usual storage temperatures, are too slow to allow small deviations from the normal course to be detected within a few days.

4.2 2.2. Deviation induced by an unknown factor

As opposed to the above a deflection may be noticed within three days if it amounts to a change from minus 0.5%/day to plus 2%/day. Such statistically significant deviations are reported by KIDD and WEST (1945) to be immediately preceded by visible decay or skin spotting. The following examples do not confirm this close relationship (see figs. 17 and 18).

In the course of three years I tested 190 apple fruits (Jonathan) for the occur-

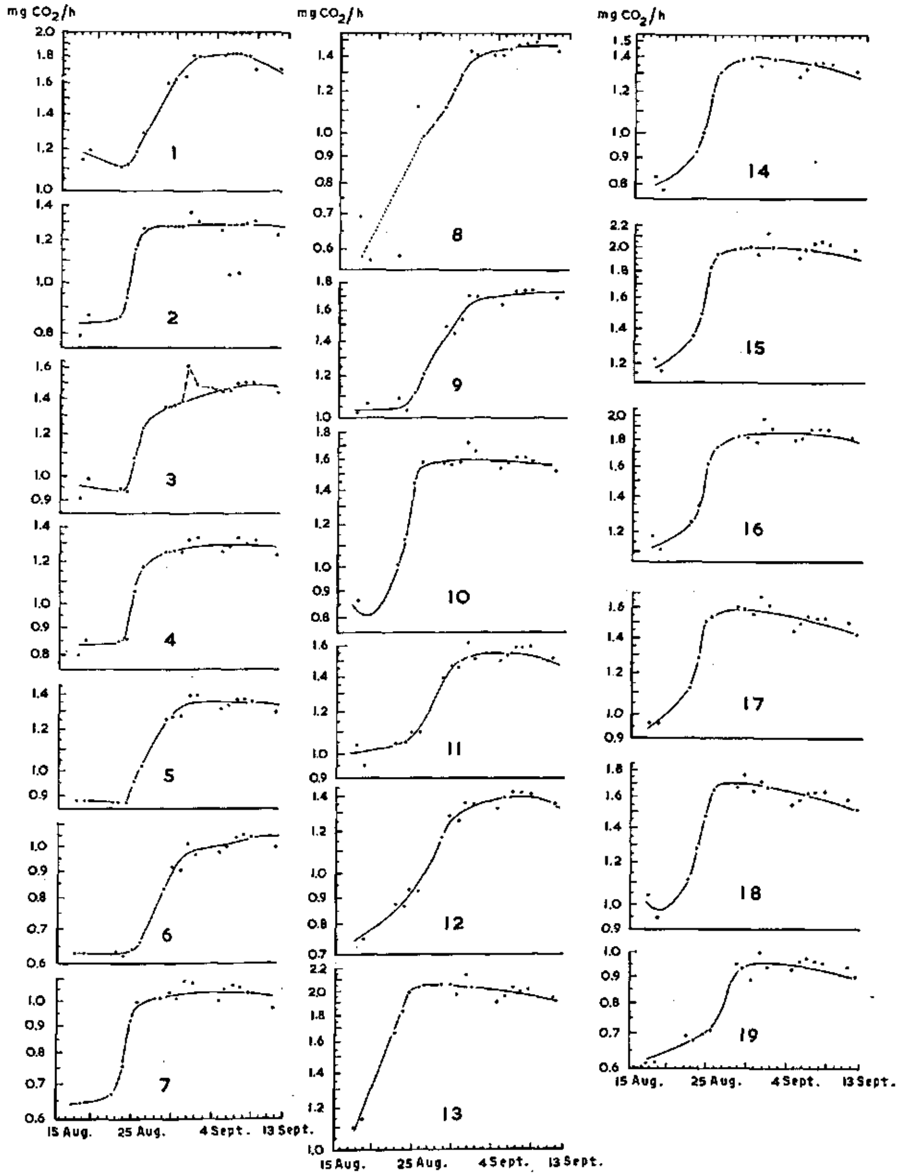


FIG. 15. Trends of CO_2-production rate at 10°C of 19 specimens of James Grieve apple picked at random from a single tree on August 14th, 1961.

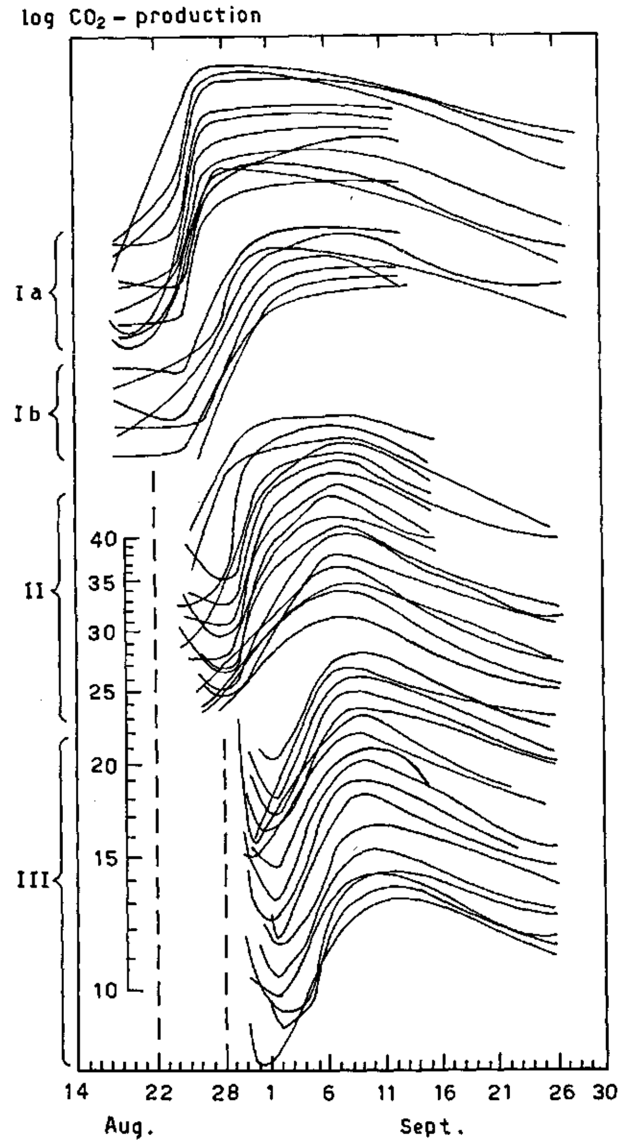


FIG. 16. Climacteric curves of James Grieve apples at 10°C, picked at random, all from the same tree, on August 14 (I), 22 (II), and 28 (III), Percentual change of respiration rate in the course of time can be read on the scale.

The curves of the first picking fall apart into two groups, the climacteric peak of the one group accurring 4 to 5 days later than in the other group; the difference has almost disappeared in the second and third pickings.

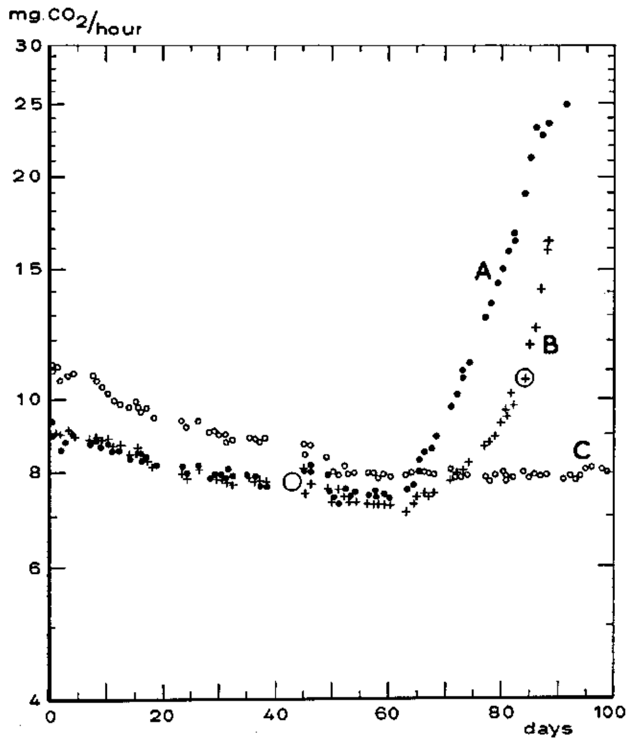


FIG. 17. CO_2-production of three specimens of Jonathan apple (A, B and C,) during 100 days' storage at 10°C (from November 14, 1960, to February 22, 1961; selected from a large number of simultaneous records). Originally, the courses of A and B were virtually identical and parallel to C. After two months, A and B began to deviate sharply from C. The deflection did *not* coincide with the onset of any externally visible deterioration. The first sign of stem-end rot on A was detected 19 days earlier (marked with circle), the first skin spotting (rot) on B appeared 22 days after the rise set in (marked with circle).

rence of (i) a more or less abrupt deviation from a rectilinear respiration-time curve and (ii) the start of some type of externally visible decay. In only 6 specimens either phenomenon occurred within a week of the other; in 31 specimens the interval was longer, 10 to 58 days. The three seasons that were studied showed unequal tendencies (fig. 18). On the average the interval was 31 days after ii in 1958–59; it was 2 days before ii in 1960–61 and 20 days before ii in 1961–62.

4.2.2.3. Different effects of a transient change of temperature

Unequal effects were found in concurrent postclimacteric courses of various lots of Jonathan and Boskoop apples. As shown in fig. 19, a transient change of temperature – at T – had a slightly delayed effect followed by an after-effect. The identical courses of Jonathan E, F and G (fig. 19) were resumed within a week after T; the same occurred with Boskoop K. In all other lots the previous

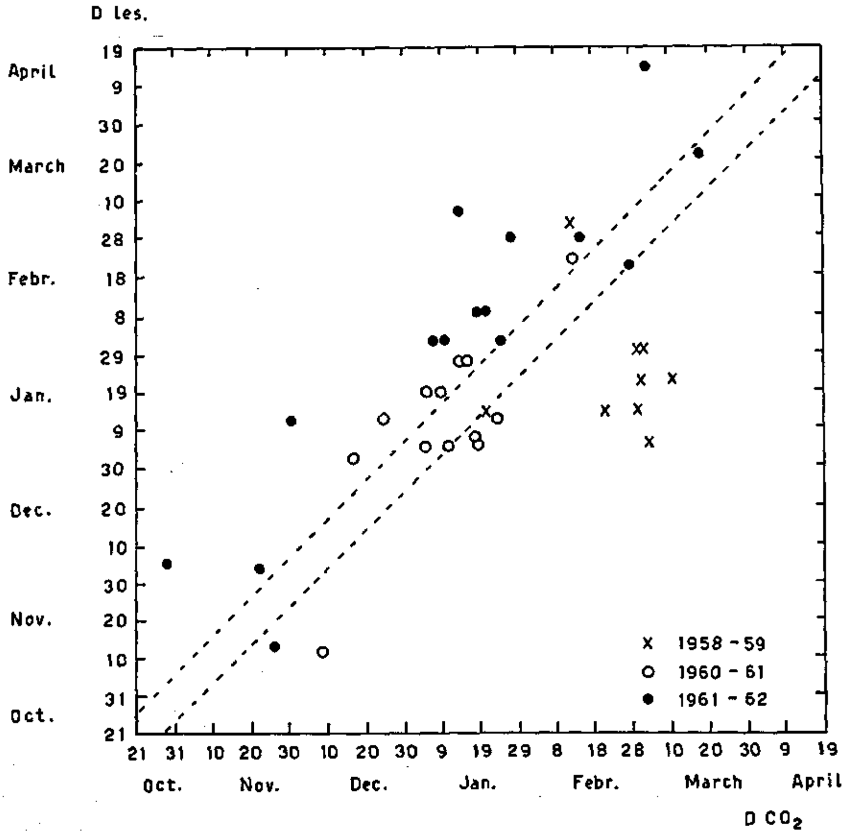


FIG. 18. Relation between the date of sudden increase in CO_2 production rate (D_{CO_2}) and that of appearance of lesions on the surface (Dles) of 37 specimens of Jonathan apple, kept at 10 tot 11°C. A time interval of one week between the two is marked with parallel broken lines showing coincidence in no more than six cases. Positions above this band indicate delayed appearance of lesions, those below the band indicate delayed rise of CO_2-production.

course was not resumed. The level had become definitely different, but the decline (% decrement/day) did not show an appreciable change, except in Boskoop D. Before T, Boskoop H, I, J and K had similar respiration rates; afterwards J and K diverged and H and I continued to be very much alike.

In either season the change of respiration rate in Jonathan started earlier and was more pronounced than in Boskoop. In Boskoop the respiration rate had not changed on the day next to T, contrary to what was observed in Jonathan. The delay may have been due to the fact that the temperature stimulus was relatively small; the change T was observed in the circulating water and was conceivably less in the apples. Contrarily, the apparently stronger stimulus discussed on p. 32 (fig. 20) was never found to lead to a permanent shift of a respiration trend.

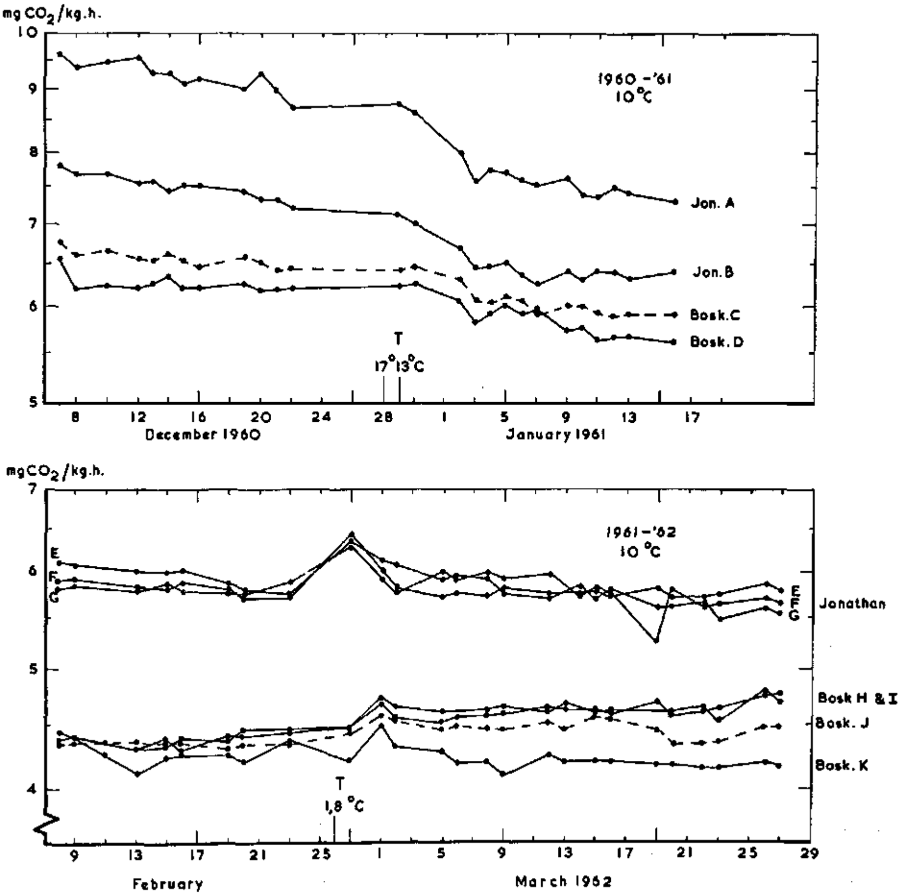


FIG. 19. Postclimacteric respiration rates of apples as affected by a transient change of temperature (at date T). Each dot represents an overall CO_2 production of a sample, as related to the sample weight at the beginning of the experiment. The samples are: *Jon. A* and *Jon. B:* 8 fruits and 10 fruits respectively of Jonathan, picked in two different orchards;
Bosk. C and *Bosk. D:* 7 fruits and 5 fruits respectively of Beauty of Boskoop that had been dipped into a solution of lecithin and sym-dimethyl-diphenylurea respectively (see Annual Report 1961, I.B.V.T., Wageningen);
Jon. E, Jon. F and *Jon. G:* 11, 7, and 9 fruits respectively of Jonathan, picked from the same tree, and transferred immediately to the respirotron on September 27, October 4, and October 11, 1961 respectively.
Bosk. H, Bosk. I, Bosk. J and *Bosk. K:* 4, 3, 5 and 5 fruits of Boskoop respectively that had been dipped into 48 % ethanol, into a solution of lecithin, into one of diphenylamine, or of sym-dimethyl-diphenylurea respectively (see Annual Report 1962, I.B.V.T., Wageningen).

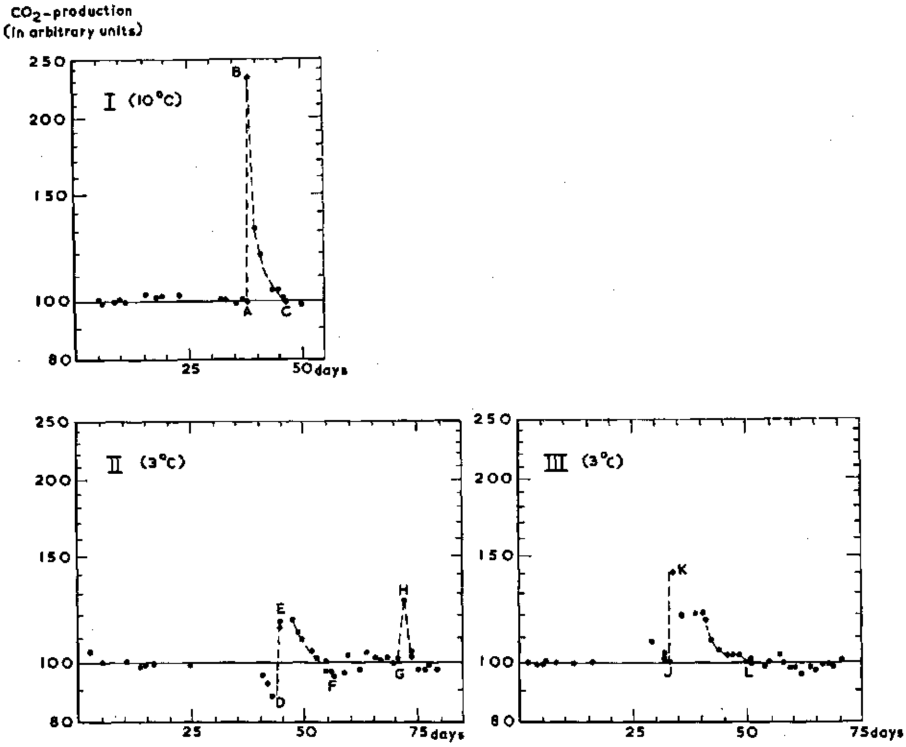


FIG. 20. CO_2 production of three Jonathan apple fruits (I, II, III). Average trend in each presumed to be 100 for the sake of comparison.
(I) At day A, a leak in the apple container was repaired. This disturbance supposed to be partially thermal caused a sudden increase up to B or possibly higher. The level 100 was re-established (at C) 8 to 9 days after A.
(II) At day D an evident reduction (12% below level 100) indicated a leak. The apple container was disconnected and the leak was repaired. This disturbance also supposed to be partially thermal, caused a sudden increase apparently far beyond level E, since level 100 was not re-established until day F, 9 or 10 days after D. Subsequently CO_2 production was rather irregular until at G a disturbance appeared that was found to be due to an obstruction of the cooling water. No after-effect appeared after the water circulation had been restored; evidently the sudden increase has not gone far beyond level H.
(III) At day J, the fruit was taken out of its container, a hypodermic needle containing a thermo-element was inserted into it and the fruit was put back into the container which was replaced into the respirotron. The next day the output was at level K, after which 15 to 16 days passed before level 100 was re-established at L. The stronger after-effect may be due to both the inserted probe and the thermal and mechanical effects of handling the specimen.

4.2.2.4. Conclusions on CO_2 production

The data given above allow the following preliminary conclusions to be drawn concerning the CO_2 production rate of single apples at 10 to 11 °C:

- the experimental error of 1 to 2% should be further reduced, otherwise small essential deviations from the normal course cannot be detected.
- a peculiar sudden increase does not coincide generally with the appearance of the first symptons of decay on the surface of the fruit. As the one effect appeared *before* the other in one season, and appeared *after* the other in another season, the two effects either have no common cause or they reflect varying host-parasite relationships.
- a small and transient thermal stimulus which is known to affect the quality of stored apples and plums may induce a change. The previous course is either resumed within a few days or weeks, or the induced deviation appears irreversible. Sometimes identical courses are affected differently; in such cases, a transient stimulus is an aid in discriminating between specimens that do not show significant dissimilarities otherwise.

5. MEASURING OF PRODUCE SOFTENING

5.1. DEFINITION OF THE PROBLEM

5.1.1. *Introduction*

Pears, tomatoes and other fruits tend to soften during storage. Softening rates are generally reconstructed from 'firmness values' obtained from different samples of a quantity of the fruit, the samples having been subjected consecutively to a certain firmness test. In such a reconstruction the overall softening trend of the fruit may be obscured by the sampling error.

'Firmness' (other terms: hardness, softness) is a sensorily perceptible relationship between a force (load) applied locally on a fruit and its deformation. The term 'deformation' applies not only to a dimensional difference as between diameters, but also to the rate at which the difference comes about. Differences in sensory dimensions – such as between pears and peaches – may be more easily understood through the use of different rates of deformation.

The sensory dimensions are evaluated by squeezing a fruit with the hand. The rule-of-thumb evaluation even if difficult to interpret has been found to accord quite well with readings from different types of measuring instruments. HAMSON (1952) found a highly significant correlation of 0.65; GARRETT et al. (1960) report percentages of agreement ranging from 35.8 to 100 (mean 75). This is the more astonishing as the principles along which the instruments operate do not seem to have anything in common with those of the squeezing hand. The latter may act in two ways that are essentially different. Either the fruit is very lightly squeezed repeatedly, or the pressure of the thumb on the fruit is increased until the fruit 'gives', at which time the thumb pressure is released (BOURNE, 1965). The 'give' of the fruit is a subjective measure of the 'yield point' that will be discussed in section 5.1.5.

In mechanical pressure testers and penetrometers a 'firmness value' may represent the deformation caused by a given load, or it may be the load required for a predeterminate deformation, or again an equilibrium position between a load and the resilience of the test fruit (ANON, 1962).

Most firmness values described in the literature are based on a standardized deformation that amounts to some type of damage done to the test fruit. These will not be considered as they do not fit the conception of non-destructive determinations aimed for in this paper.

5.1.2. *Semi-destructive firmness tests*

Firmness tests based on the deformation caused by a given force, are described by HAMSON (1952) and KATTAN (1957). HAMSON uses two parallel surfaces, a plunger and a platform, between which the test fruit is slightly compressed. In KATTAN's method the test fruit is pulled against a wooden block by means of a chain partly constricting the specimen. Comparing his readings with those obtained with the HAMSON method KATTAN found a correlation of

92.3% and concluded that the two instruments are equally dependable in measuring firmness.

HOBSON (1959) modified KATTAN's method in order to avoid damage to the specimen. Neither HOBSON's version nor HAMSON's method are to be considered non-disturbing to fruits; both methods necessitate a test fruit to be removed from storage conditions for transfer to a measuring instrument.

5.1.3. *Prerequisites for measuring softening*

In order to determine individual rates of softening of fruits in storage, each test specimen should be equipped with a device that allows the application of a deforming force and the evaluation of the deformation. A given load, applied to a fruit, causes a deformation of the fruit and a displacement of some part of the device. A plunger moving in a straight line may cause the tissue of the test fruit to move both inward and sideways. In other words, 'practical correlation' between the two may be low (MOHSENIN, COOPER and TUKEY, 1963). A possible lack of practical correlation does not necessarily prevent softening from being evaluated, provided a greater deformation of the test fruit is indicated by a greater deflection of the device.

Any proposed device must be fixed in such a way that it cannot be displaced unintentionally because in tomatoes (HAMSON, 1952) as well as in apples and pears (KRUMBHOLZ and WOLODKEWITSCH, 1943) different parts of the surface of the fruit may show (widely) different 'firmness values'. Although a series of firmness values derived from a restricted part of the surface of a test fruit may not be considered representative of the overall softening trend in the specimen, these values will allow comparisons to be made between fruits and in this way may be of value in detecting anomalies during storage.

HOBSON (1959) obtained an approximation to an 'overall firmness value' by taking the average of four readings made with the specimen (tomato) in different positions. He reports the average to be more consistent if the first of five readings is discarded. A similar observation is recorded by MOHSENIN and GÖHLICH (1952) who loaded an apple fruit until a little below its yield point (to be discussed below), unloaded it, and repeated the cycle several times without changing the position of the test fruit. The first of the successive force-deformation curves appeared to be quite different from the others which were of about equal shape.

In tomatoes, the difference between the first and subsequent deformations may depend upon the direction of the deformative force. The present author observed this, using a method closely resembling HAMSON's and taking each reading after the force had acted for one minute. The first of the three successive readings differed 20% from the other two when the direction of the force was at right angles to 'a straight line extending through the fruit from the stem scar to the blossom and scar' as in HAMSON's and HOBSON's apparatus. When the force acted along the said line the observed difference was about 5%.

A test specimen installed in a device which combines a deformative force and a deformation-sensitive element may be expected to show consistent readings after the first determinations have been made and the fruit as somehow 'settled' for the remainder of the storage period.

5.1.4. *Static load*

A deformative force may act continuously, intermittently, by impact, or by vibration.

A continuous force of unchanging strength and direction (static load) will cause deformation to proceed at a rate depending on the rheological properties of the test fruit. The tendency of fruits to be gradually deformed when packed tightly is well known among commercial shippers. If too high, a static load causes internal lesions (McCOLLOGH, 1962; MOHSENIN and GÖHLICH, 1962). Whether a static load, not high enough to be injurious, will cause a deformation to proceed throughout a normal storage period is not known. As may be derived from a time-compression curve presented by MOHSENIN, COOPER and TUKEY (1963), the rate of compression of an apple after 170 hours static load may be in the order of 0.02 mm/24 hours.

5.1.5. *Yield point*

As defined by ANON (1963), the 'bio-yield point is the change in slope of the force-deformation curve at which cell rupture takes place immediately under the skin. In certain fruit, such as apples and pears, the bio-yield point is demonstrated by a sharp break in the force-deformation curve as a result of an increase in deformation with a decrease or no change of stress.' The latter refers to the fact that the tissue may collapse under static load (no change of stress) after several hours, or even days. The bio-yield point then appears as a break in the time-deformation curve (MOHSENIN and GÖHLICH, 1962). A series of comparable yield point values of apples of different varieties and after different periods of storage covered a range from 0.5 to 1.2 mm. The determinations were performed on apples that were cut along their main axis; each half of a fruit was then compressed between two parallel plates. Apparently, *whole apples* between parallel plates should not be compressed more than twice the minimum value, i.e. one millimeter, to avoid damage. The above authors have shown that a force-deformation curve of a *tomato* is a straight line or a slightly undulating one up to the point of rupture of the fruit's skin. This suggests that in a tomato a yield point does not exist, or that the test fruit has been bruised previously, as yielding is supposed not to repeat itself. In view of this possibility, certain precautions in testing tomatoes seem imperative (see 5.3.3) as bruises in tomatoes do not show as readily as the resulting deformations and discolorations in apples.

Another consequence of the non-existence of a yield point in a tomato would be that any deformation short of rupture of the skin would be reversible. In view of this possibility a series of tests has been made as described in 5.3.3.

5.1.6. *Compression and shrinkage*

The yield point of apples seems to be independent of the rate at which it is attained. This may be concluded from MOHSENIN and GOEHLICH (1962) reporting that in static load tests the yield point may occur either after a few seconds or after several days; the shape of force-deformation curves is not affected by the rate of compression within a range of 0.5 to 25 mm/min. At compression rates of 25 to 330 mm/min, force-deformation curves are identical below one millimeter compression.

This implies that one millimeter compression is a limit in non-destructive static load tests on apples. Consequently in a long-term test lasting a hundred days, differences in softening rates would have to be read from decrements of about 0.01 mm/day. Although this is technically feasible (see section 5.1.7) decrements of much more than 0.01 mm/day are to be expected as a result of normal shrinkage due to moisture loss. Moisture loss has been reduced by exposing the test fruits to an atmosphere of low temperature (MOHSENIN and GÖHLICH) and/or high humidity. A high humidity, however, tends to swell a fruit (WIKINSON, 1961) and sometimes causes subepidermal accumulation of water (GAC, 1955), both factors interfering with 'normal softening'.

All this implies that it does not seem advisable to enter into long term tests involving a static load.

5.1.7. *Magnification of minute compressions*

Compression in the range of zero to one millimeter cannot be measured exactly without being magnified. As explained in chapter 2 a convenient scale for routine measurements should offer at least 100 mm difference between the highest and the lowest values expected; consequently, non-destructive deformations in apples should be magnified at least a hundred times.

Compressions performed with the aid of a METTLER precision balance (see section 5.2.2) are optically enlarged about three hundred times. Linear differential transformers as used by MOHSENIN (1963) are considered to measure displacements of 10^{-6} inch which implies a ten thousand times magnification. With the aid of resistance strain gages MOHSENIN (1963) obtained a thousand fold magnification.

As will be described in section 5.2.4 hydraulic transducers may lead to magnifications of 200 to 3000 times. It is because of the almost unlimited possibility of enlarging periodic deformations, with the aid of mechano-electric transducers, that mechanical vibrations are of considerable interest in this type of work; this aspect is discussed in section 5.1.13.

5.1.8. *Influence of diameter*

HAMSON (1952) considered the possibility that a large fruit would be compressed to a greater extent than a small fruit of equal firmness.

He was able to refute this assumption in comparative measurements on tomatoes. The fruits were compressed a few millimeters and the correlation between firmness and diameter was no better than 0.037. Similarly non-destructive compressions of apples may be assumed to be totally unaffected by the size of the test fruit.

5.1.9. *Limited range of action of compression tests*

The above conclusion indicates that non-destructive compression tests apply to a very restricted part of the test fruit. MOHSENIN, GOEHLICH and TUKEY (1962) found that in apples that had been compressed over their yield point, the bruised area was 'exceedingly small and located in the flesh near the skin'. Away from the area of contact of a deforming force, the dislocation of fruit tissue apparently diminishes rapidly, the more so if the deforming force is vibrational. On the other hand, the loss of vibrational energy is negligible compared to the tremendous gain inherent in the mechano-electric magnification of vibrational deformations (NYBOM, 1962).

Because of its limited range of action, a non-destructive compression test may fail to show certain characteristics of the 'overall firmness' of fruit. As a conclusion of his work on tomatoes in a breeding program, HAMSON (1952) states: 'A characteristic firmness of the fleshy tissue of fruits of most firm lines appeared to be far more important in conditioning firmness than such fruit characteristics as skin strength, thickness of outer and inner walls, proportion and structural arrangement of wall tissues, number of locules, and size of fruit'. HAMSON found no more than –0.8% to + 9.0% (mean 4.0%) difference between the average values of compression of tomato fruits that were loaded over a locule or over a crosswall. The same observation is discussed in section 5.3.4.

5.1.10. *Intermittent loading*

Intermittent loading and unloading causes alternative compression and recovery (retraction), the amplitude and duration of which can be adapted to the purpose envisaged. Compression and retraction can be related to the force applied (which may be increased and decreased at an arbitrary rate), or to the time (an arbitrary load being applied at once, or removed at once).

Force-deformation relationships are discussed by MOHSENIN and GOEHLICH (1962). With the aid of a plunger moving at a rate of about 4 mm/min they found in apples a nearly straight line during loading (to just below the yield point) but not during unloading to zero stress; alternative loading and unloading resulted in a hysteresis loop. The area encompassed by this loop is said to represent the hysteresis loss plus a small amount of permanent deformation due to the plasticity of the fruit. In the first of a series of loading-unloading cycles, performed on a test apple, the hysteresis loss was found larger than in further cycles, in which losses were about equal.

The same difference between the first and successive tests has been observed in the time-deformation relation of tomatoes, as reported by HOBSON (1959), and in unpublished investigations of the present author. In both cases this has led to the practice of basing 'firmness values' on the results of second and successive tests. In the tests, the time-deformation relationship of fruit is determined in its simplest form: the compression is read after an arbitrary time under an arbitrary load, the data obtained being used as 'firmness values'. This simplification, although satisfying practical demands, does not suit our present purpose, because it disregards the additional information obtainable from time-deformation curves. It also may lead to serious inaccuracies if the readings of compression are taken after too short a loading period. HAMSON (1952) reduced the time interval to five seconds, HOBSON (1959) to thirty seconds.

According to our experience (see section 5.3.1.), a tomato, subjected to a compressive load of 500 grams still deforms perceptibly after half a minute. This 'creeping deformation' necessitates a test to be discontinued (or reversed) at a well-chosen moment. As will be shown in section 5.3.7 useful information on rheological properties of fruit may be obtained by means of loading-unloading cycles of a few minutes duration.

5.1.11. *Load changing inversely to compression*

After a load has been applied or removed, the initial rapid deformation gradually slows down to creeping deformation. The latter decreases to the magnitude of normal shrinkage. This ultimate rate can be reached within a shorter time, if a load is made to decrease during compression, and to increase during recovery. This had been realized as follows. ANON (1962) describes an apparatus, consisting of a METTLER Precision Balance ('Präzisionswaage'), to the pan of which a horizontal bar is attached. The other end of the bar is provided with a circular plunger, diameter 7 cm. A test fruit (tomato) is placed on a platform alongside the balance. The platform is screwed up or down until the fruit just touches the plunger. The balance pan is loaded with a weight of one kilogram, which causes the pan to come down and the plunger to compress the fruit. The lowering of the balance pan is read on the balance scale; a reading of 110 gram corresponds to one millimeter. Thus the compression of the fruit can be read as a weight value on the scale. The greater this 'firmness value', the softer is the fruit.

In private correspondence, the originator of the method, Ir. M. HERREGODS, of the State School of Horticulture, Vilvoorde, Belgium, added that the 'firmness value' was read after five seconds compression.

Evidently, during the downward movement of the plunger, the load on the plunger decreases at a rate of 110 gram per millimeter. The decreasing load meets an increasing resistance or resilience of the test fruit. Any 'firmness value' read after an arbitrary period of compression depends on a combination of variables, part of which are bound to the particular apparatus used; the result might be closer to an ultimate value than it would have been under constant load. This assumption has been tested as described in section 5.3.1.

5.1.12. *Impact loading.*

The effect of an impact – a force applied instantaneously – may be observed when a box of fruit is poured out on the soft surface of a sorting table. The collisions of the fruits cause a dull, shuffling noise. The character of the noise differs with the consistency of the fruit: when firm Jonathan apples collide, high-pitched sounds, although barely audible, definitely stand out against the dull background noise. We never perceived such sounds with softer or more pulpy fruits, like pears or tomatoes.

Likewise a single fruit tapped very lightly, for instance with a finger nail, will produce a barely audible noise which allows the same distinction: a firm apple produces a distinct sound that is lacking in a softer one.

Any impact on a fruit induces complicated and heavily damped vibrations in the specimen (MOHSENIN and GOEHLICH, 1962), causing some pattern of waves to be transmitted to the human ear. Evidently the effect depends on the 'consistency' of the test fruit as well as on the strenght of the impact. This phenomenon is useless however as an indicator of the softening of fruits under storage conditions since it necessitates listening with the ear close to the test object; it probably is induced mainly in a small part of the skin, the area of impact, and the influence of the 'firmness' of the fruit may be small. Even if the effect would change with a diminishing firmness, the relation would be too complex for our purpose (see 5.1.13.1, resonance).

Another effect of an instantaneous force is the pulse echo (McGONNAGLE, 1961). With the aid of a suitable electro-mechanical transducer, a very short sequence of ultrasonic waves is sent into the specimen. Because of reflection at the opposite face and at internal inhomogeneities of the specimen, ultrasonic waves travel all through the object, losing part of their energy in heat conduction, viscous friction, elastic hysteresis, and scattering. Part of the reflected waves are picked up by the transducer and can be studied with the aid of a cathode ray oscilloscope. The echo pattern may be too complex for routine measurements of softening rates in fruits, although it is known to be an important aid in medical diagnosis (BEIER and DOERNER, 1956).

5.1.13. *Vibrational loading*

An alternating force, inducing periodic deformations (forced vibrations) in a test object, can be applied to find the test specimen's resonance frequencies (using vibrations of varying frequencies), or to measure energy losses due to the absorption in the specimen.

5.1.13.1. Resonance

Resonance frequencies, in relation to the consistency of vegetable tissue, have been studied by VIRGIN (1955) and FALK, HERTZ and VIRGIN (1958) in the following way. A small rod of potato parenchyma was clamped at one end, allowing a length of about 8 mm to swing freely. At different frequencies, swinging was induced by an alternating magnetic field actuating a small piece of detempered steel that had been attached to the free end of the parenchyma rod. The resulting amplitudes were observed through a microscope. At some frequencies the rod would swing more easily (widely) than at the applied next higher and lower frequencies. The numerical values of the outstanding (resonance) frequencies showed mutual proportionalities from which the fundamental resonance frequency (about 70 to 80 cycles per second) could be calculated; it was found to depend on the turgor of the parenchyma tissue.

Like a small rod of potato parenchyma (as well as wheat root and a leaf of

Helodea densa, all three simple-shaped test objects being discussed by VIRGIN) a fruit can be made to vibrate when an alternating force is applied to it. However, the simple relationships VIRGIN found between turgor and resonance frequencies cannot be expected in whole fruits owing to their complicated shape and their heterogeneous structure.

This negative postulate could be confirmed in a series of experiments the present author made on tomato, apple and pear, as well as on dummy fruits that were made to change their rheological properties within an hour. Similar test objects have been used in the investigations described in section 5.2.5.2.

5.1.13.2. Transmission

NYBOM (1962) measured rates of transmission of 50 Hz vibrations through raspberries and found them to decrease with increasing softness of the fruits.

However there are some reasons to doubt the validity of NYBOM's method if applied to measuring fruit softening during storage.

NYBOM used radio receiver earphones of about 5 cm diameter as a vibrator an as a pickup (contact microphone).

When placed against opposite sides of a test fruit, these telephones obstruct visibility of the greater part of the surface of the fruit. A smaller type with 1.5 cm diameter, which is commercially available might be used for the study of softening in relation to external characteristics. With a small telephone membrane like that a shift of a few tenths of a millimeter of the area of contact with the test fruit strongly affects the energy transmission, as found in our work on resonance in fruit. If the telephone membrane were affixed to the fruit skin with glue, the underlying tissue would be badly affected. If necrosis could be avoided, a change in vibrational energy transmission might be due to softening of the test fruit, to slackening of the skin or to shrinkage.

These considerations tend to reduce the usefulness of vibrational energy transmission as a tool in measuring softening.

Mutual interference of the effects of softening and shrinkage is also to be taken into account when using the hydraulic transducers described in section 5.2.4. The effect of shrinkage might be estimated from concurrent determinations of weight loss and transpiration of the test fruit.

5.1.14. *Support*

In nondestructive firmness tests, a suitable support of the test fruits is of sufficient importance to give it some special attention.

MOHSENIN and GOEHLICH (1962) describe the appearance of two yield points in an apple that was compressed between two plungers. The same may be expected when compression is performed between two flat plates, or between a plunger and a platform, each of which provides a yield point.

A virtually one-sided compression is attained if the pressure at the opposite side is spread over a larger surface of the test fruit. To that end MOHSENIN and GOEHLICH (1962) performed compression tests on a segment of a test fruit (apple) that had its cut surface smoothed with sand paper and placed on a flat surface. ANON (1963) mentions a plaster of paris cast, which is said to result in a more uniform stress distribution.

NICHOLAS (1960) placed the test fruits in 'a sand-filled Petri-dish'. But I found – section 5.2.5.1. – that a test fruit, if merely placed on a sand support, may sink as much as one millimeter deeper into the sand while being compressed.

5.2. METHODS

5.2.1. *Introduction*

Among the different methods mentioned, none seems entirely satisfactory for measuring softening rates of fruits in store. The ideal method would have to comply with mutually conflicting requirements. This is why concessions were necessary in each of the methods described in the next pages. One of these concessions is that no attempt will be made to express test results in terms that would satisfy physical or technical considerations. For instance, because of the non-uniform curvature of a test fruit, a deforming force cannot be said to amount to x kg/cm^2 and a deformation cannot be said to amount to y mm on the average in a certain direction. Instead, we can only express our results arbitrarily as a linear displacement observed in a transducer in close contact with the test fruit. In the methods to be described, the measured displacement was magnified at least two hundred times. Amplified in this manner compression can be kept well below the yield point of the fruit.

Only two modifications called 'hydraulic swellers' (see section 5.2.4.) apply to some requirements of nondisturbing observation as mentioned previously. The other methods necessitate the removal of test fruits from storage conditions. and can be used only as a non-destructive check. A check is necessary, both at the beginning and at the end of the experimental storage period, as any change observed with the aid of 'hydraulic swellers' may be due to softening as well as to shrinkage.

5.2.2. *Mettler compressor*

Three types of METTLER Precision Balances ('Präzisionswaage') were tried, viz. P-10, K-5-T and P-120[1]. In these balances a lowering of the pan by one millimeter is read as 1322, respectively 132.1 and 1.878 grams shift of the optical scale. This implies a linear magnification of respectively 593, 360 and 335 times.

With the aid of these balances, the method originally described by ANON (1962) has been modified as follows (see fig. 21).

A short brass rod was used as a plunger. One end was screwed into the nut at the lower side of the balance that can accomodate a suspended load. The other end of the rod protruded 2 cm below the bottom of the balance; its flat surface, 10 mm diameter had its edge ground to a radius of 0.2 mm. The balance feet rested on a three-legged support of 10 cm height.

In order to make the plunger 'just touch' the test fruit the plunger was lowered, partly by manipulating the tare mechanism of the balance, partly by

[1] By courtesy of the METTLER importer in Holland: N.V. Dr. D. H. COCHERET, Arnhem.

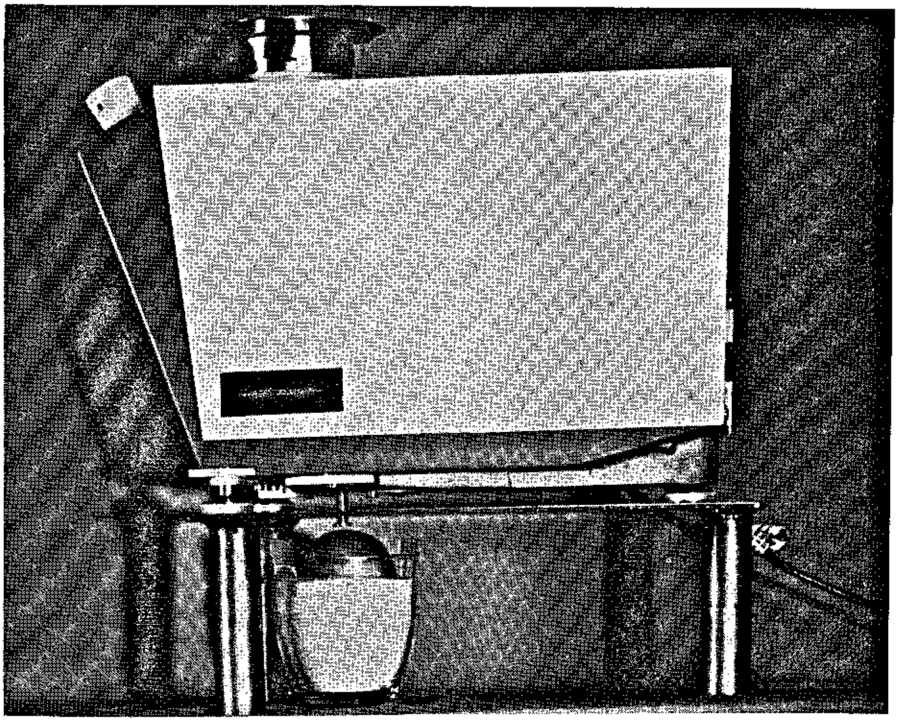


FIG. 21. METTLER Compressor.
Test apple is partly immersed in fine dry sand in a glass tumbler. Two diametrically opposite "ears" (made from adhesive tape) are fixed to the rim of the tumbler; they serve as a guide to the right depth of immersion of the test fruit. Almost touching the top of the fruit is the plunger, that protudes from the balance (METTLER P-120 type) through a hole in the three-legged support of the balance.

increasing the load on the balance pan. A small increase of the load caused the balance scale to shift. As long as the plunger moved freely, the shift corresponded to the added weight. But as soon as the plunger touched the test fruit (even a very soft tomato), the shift of the scale would lag behind. With a little practice, the beginning of this lag could be distinghuished from the effect of the magnetic damping, which was especially strong in P-10 and in K-5-T.

Under the plunger of a METTLER Compressor, a working space of 10 cm height was available to the test object. This height could be adjusted no more than a few millimeters. Greater adjustments necessitated the test object to be placed on a support that allowed a wide variation of heights, and that was virtually incompressible, and provided a large area of contact with the test fruit.

To meet these requirements, the sand support was used that is described in section 5.2.5.1.

5.2.3. *Bellows Compressor*

As shown in fig. 22, this device consists essentially of a bellows, one side provided with a plunger, the other side fixed to a frame and connected to a piece of transparent flexible tubing. The bellows and the tube are filled with a liquid of low viscosity. Extension or contraction of the bellows changes the position of the meniscus in the transparent tube; any change of the hydrostatic pressure in the bellows tends to move the plunger.

The dimensions of the bellows in relation to the inner diameter of the manometer tube determine the sensitivity of the apparatus; the flexibility of the bellows determines the working range of the plunger.

Suitable dimensions and flexibility were found in a plastic salt sprinkler, a household utensil that is commercially available in this country. Its domed plastic screw cap made an excellent plunger, although it was perforated. A liquid-tight closure was obtained by means of a rubber stopper, pressed firmly into the neck of the bellows with the cap screwed over it. A bicycle tire valve was inserted through a hole in the bottom of the salt sprinkler; its valve function was eliminated as the tip of its inner tubule had been clipped off. The manometer tubing (two meters of clear transparent polyvinyl chloride, internal diameter 2 mm) was fixed to the inner tubule. The outer tubule was cemented to the movable jaw of a vernier caliper. Also cemented to the movable jaw was a rigid plastic tube which was provided with a one-meter scale and which was slipped over the rod of a laboratory stand. The other jaw of the vernier caliper rested on the base of the laboratory stand and had a small platform rigidly fixed to it to accomodate the test object. The distance between the movable jaw and the other jaw of the vernier caliper was adjusted until the bellows plunger just touched the top of the test object. When suitably adjusted the movable jaw was secured to the stem of the vernier caliper. The extent of an adjustment could be read on the vernier scale of the caliper. Otherwise the vernier scale was of no use in compression tests.

To make the apparatus ready for use the bellows and the manometer tube were filled with ethanol free from air-bubbles. The manometer tubing was then held in such a position that the meniscus was level with the zero reading of the one-meter scale. Etanol was added or removed until the meniscus when held at the zero level of the one-meter scale was 20 cm from the open end of the manometer tube. In that position the manometer tube was clamped onto the scale and the common level was marked 'zero' on the transparent manometer tube.

To prepare a firmness test, a test object was placed on the small platform and the movable jaw of the caliper (bearing the bellows and the manometer tube clamped onto the one-meter scale in the 'zero' position) was fastened on the caliper stem, the bellows plunger just touching the test object. This condition could be readily established, as the slightest compression of the bellows would induce an appreciable shift of the meniscus (The relation existing between compression and shift will be discussed later).

With the bellows plunger in ultimate contact with the test object and the

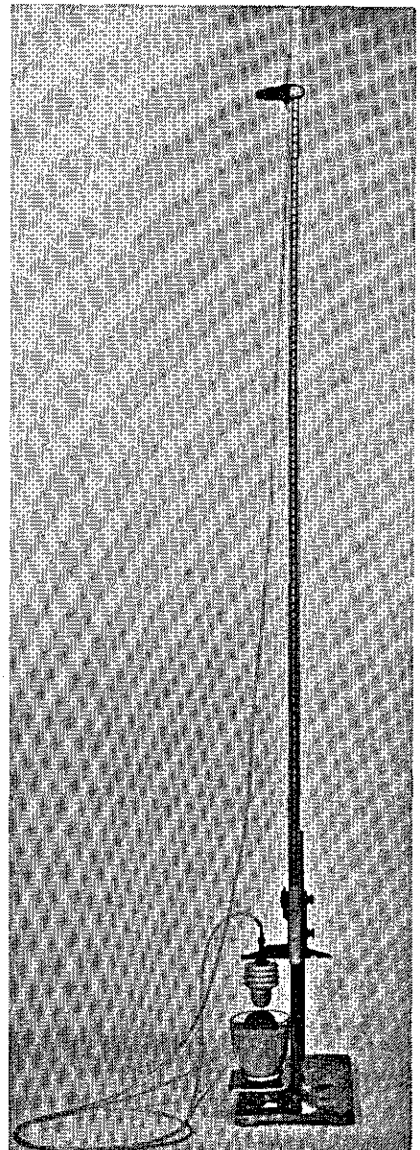


FIG. 22. Bellows Compressor.
A length (1.2 meter) of rigid plastic tubing is provided with a metric scale (SCALAFIX, London, England) of 1 meter and is slipped over the stem of a *laboratory stand*. The lower end of the tube is fixed to the movable jaw of a vernier caliper. A plastic bellows (an inverted salt sprinkler with a bicycle tire valve screwed in its bottom) is also fixed to the movable jaw. A metal platform is fixed to the other jaw of the vernier caliper that rests on the base plate of the laboratory stand. On the platform is a glass tumbler filled with fine dry sand and with a test fruit partly immersed. A length (2 meters) of flexible transparent tubing (2 mm bore) is connected to the bellows, partly filled with manometer liquid (ethanol) and clamped to the top of the rigid tube bearing the metric scale. The zero level of the scale is 10 cm above the bottom of the bellows.

movable jaw fixed with the aid of the set screw, the manometer tube was unclamped and its open end pulled upwards until the 'zero' mark was level with the 'one meter' reading on the one meter scale. In that position the manometer tube was again clamped onto the scale.

Pulling the zero mark up one meter will be called the 'standard manipulation' of the apparatus.

The increased hydrostatic pressure caused a certain volume of manometer liquid to flow into the bellows, while the bellows extended and the meniscus in the transparent tube fell accordingly.

If no test object opposed the extension ('free extension'), the resilience of the bellows alone would stop the inward flow of alcohol after the meniscus had come down to a certain level. This 'zero firmness value' depended upon the density of the liquid and other characteristics of the device.

If the standard manipulation was performed after the bellows plunger had been put into contact with the platform of the device ('blocked extension'), the inward flow of liquid would stop at a higher level, to be called 'infinite firmness value'. This again depended upon the density of the liquid and other characteristics of the set-up.

If the standard manipulation was performed after the bellows plunger has been put into contact with a compressible test object, the liquid meniscus would come down to a level between 'infinite firmness' and 'zero firmness'. This level, represented a 'firmness value'.

Test fruit firmness values were found directly proportional with the height to which the manometer tube was lifted. In 27 compression tests performed on the equator of 5 tomato fruits, hard, medium and soft, the manometer tubing was first lifted up to one meter and after 30 seconds a first reading (f_1) was taken; the manometer tubing was then pulled up 0.5 meter more, and after another 30 seconds a second reading ($f_{1 \cdot 5}$) was taken. The 27 data of f_1 ran from 42.9 to 58.7 cm, the ratio of corresponding $f_{1 \cdot 5}/f_1$ values was 1.51 $\pm$ 0.01. This direct proportionality allowed the conclusion that in comparative work on fruit the manometer readings could be used as 'firmness values', without previous correction, that is without subtracting a zero firmness value.

To find the relation between a certain fall (F) of the meniscus (resulting from standard manipulation), and the depth of penetration (E) of the bellows plunger into a test object, a series of model experiments was made: the standard manipulations were performed after the bellows had been fixed at different levels above the platform of the apparatus. The first level kept the plunger in direct contact with the platform, so the 'infinite firmness' value was read; the next were 0.1 mm, 0.2 mm, 0.3 mm, etc., higher, allowing increasing elongation of the bellows and resulting in a series of meniscus positions. The step by step fall of the meniscus showed that F/E was 310 $\pm$ 6.4 ($N = 2 \times 14$). The maximum elongation, which was the 'free extension' of the bellows, was not attained until the bellows had been raised 1.4 mm; in other words the free extension amounted to 1.4 mm. In 12 more readings, the 'infinite firmness' value was 815 $\pm$ 2 mm and the 'zero firmness' value was 387 $\pm$ 4 mm. Evidently, standard manipulations would never cause a test object, however soft, to be compressed more than 1.4 mm, and however hard, to be loaded with more than the equivalent of 815 mm ethanol.

The relation $f_{1.5}/f_1 = 1.51 \pm 0.01$ shows the coefficient of variation to be 0.7%, whereas the relation $F/E = 310 \pm 6.4$ suggests a 2% variation. The greater variation may be due to inaccurate reading of the vernier scale of the bellows apparatus; it is of no consequence because in standard bellows manipulations the vernier scale is not read. The estimated standard deviation of the series shown in fig. 33 is about 0.004 mm.

The compression resulting from standard manipulation might be considered too much for small and delicate fruits, or it might be too small to attain the yield point. A smaller load could be applied simply by lifting the zero mark of the manometer tube to less than one meter. To attain a greater load (equivalent with the pressure of one meter water, and more) a variable air pressure was applied to the open end of the manometer.

To allow convenient reading of a shift of the meniscus, corresponding to more than 1.4 mm elongation of the bellows, the full length of the manometer tube was laid along an almost horizontal, slightly slanting scale. In that way, the rate of displacement of the liquid level could be observed over a distance of about two meters, while a steady increase of the air pressure on the open end of the manometer was applied up to the equivalent of a few hundred millibars. This was achieved by connecting the manometer tube to an air-in-water overflow system, while the water level was made to rise with the aid of a virtually constant inflow of water.

If a hard test object was loaded that much however, the bellows would bulge to one side which rendered it useless for measurement. Such a low limit is not set to the METTLER Compressor; this can be loaded with the equivalent of several atmospheres which is more than will ever be needed in nondestructive testing of fruits.

As has been demonstrated in fig. 33, 'zero firmness' and 'infinite firmness' readings were both subject to drift; they decreased 8 ± 2 mm ($N = 9$), when the bellows were used for a few hours in succession but they would regain their original value after a day's rest. This type of drift suggests that the handling made the bellows more extensible, as an effect of thixotropy and/or an unnoticed rise of temperature.

5.2.4. *Hydraulic Swellers*

The standard bellows method, however simple and precise, necessitates a test fruit to be transferred from storage conditions and to be placed in a certain position relative to the plunger of the bellows. This procedure, especially when performed again and again, does not fit in our conception of non-disturbing investigations. Hydraulic swellers were found to suit that purpose appropriately.

A transducer of this type consisted essentially of a length of non-rigid nylon lay-flat tubing (PORTEX, F 1/4 A, thickness 0.02 mm, width 6 mm when laid flat), closed at one end, the other end connected to a nylon capillary (PORTEX

N 00, 0.5 $\times$ 0.63 mm, length at least one meter). The slack tubing and part of the capillary were filled with a manometer liquid (e.g. kerosene, boiling range about 190 °C, coloured with a Sudan dye). At the open end of the capillary tube an adjustable air pressure could be applied, which forced the manometer liquid into the slack tubing and caused the liquid level in the capillary to move accordingly. At a given pressure, the movement of the liquid level was fastest if the flat tubing was allowed to swell freely. When the tubing was suitably coupled to a test object, its swelling (deformation) was counteracted by the rheological properties of the test object, and the rate of inward flow was reduced accordingly. As will be specified below, transducers of this type could be coupled to the surface as well as to some part inside the test fruit.

In the case of increasing counteraction (resilience) of the test fruit, the movement of the liquid level in the capillary slackened gradually (creeping deformation). Next, a suitable reduction of the air pressure at the open end of the capillary caused the movement to reverse, as it allowed the test fruit to recover from the preceding compression. This recovery was virtually unhampered, unlike the recovery under a plunger that is retracting at a predertemined slow rate (as in the apparatus described by MOHSENIN, 1963). Compression and recovery could be studied while the test fruit was enclosed in a container in a constant storage atmosphere, and while the manometer liquid level was somewhere outside the container.

When a flat transducer starts swelling, the two opposite walls will move mainly in a plane-parallel way. In a flat transducer of 6 $\times$ 20 mm^2 that takes 1 cmm of liquid, the opposite surfaces will come apart 1/120 mm while the liquid level in the capillary (0.5 mm bore) moves 1/0.2 or 5 mm. In this example, the transducer deformation is magnified 600-fold; if transducers of a larger size and capillary tubes of a narrower bore are used, magnification may amount to several thousand times.

A flat transducer, the swelling of which is counteracted, tends to stretch lengthwise and width wise. A certain ratio found between applied pressure and induced movement (of the liquid level in the manometer tube) may not exactly represent the test fruit's deformation: but if a transducer is coupled to a test fruit, any change in the mechanical and rheological properties of the compound may be expected to induce a change in the relation between pressure and movement.

In the preparation of this type of transducers the sealing of a nylon capillary on to slack nylon tubing was found difficult, even with the aid of an impulse heat sealing equipment. Most of the seals made were unsatisfactory, as they either impaired the movement of the manometer liquid or showed leakage when submitted to a pressure of a hundred millibars.

Transducers were prepared for either internal or external application. Those intended to be applied inside a fruit were 2 to 4 cm long. With the aid of a hypodermic syringe connected to the capillary tube they were filled with manometer liquid, made free of air bubbles, and flattened. They were inserted, in the way

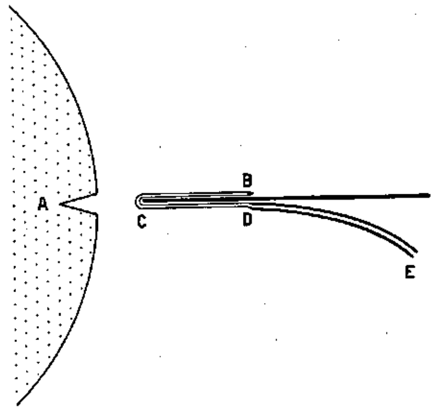


FIG. 23. Insertion of a pressure transducer (hydraulic sweller) into a test fruit. A: shallow slit cut in test fruit to facilitate insertion. B-C-D: slack nylon tubing, flattened and folded over the square tip C of hard steel strip F-C (length 100 mm, width 6 mm, thickness 0.25 mm); after insertion to a suitable depth the steel strip F-C is withdrawn, leaving the folded transducer inside the fruit. D-E: capillary connecting tube.

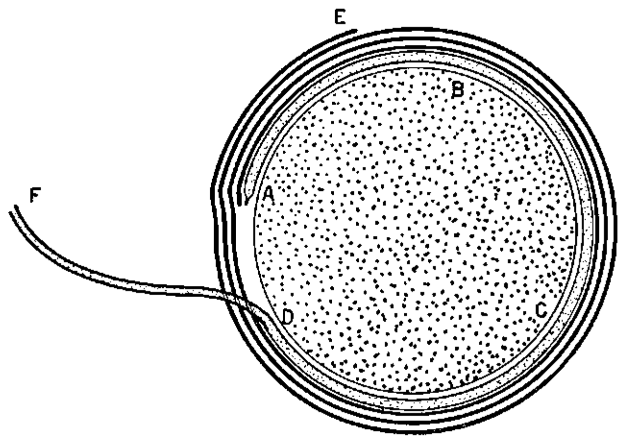


FIG. 24. "Hydraulic belt".
A-B-C-D: slack nylon tubing filled with manometer liquid, one side pressed against test fruit, the opposite side glued onto adhesive tape A-B-C-D-E, which is reinforced with glass fibres. D-F: capillary connecting tube going off to one side.

shown in fig. 23; the slack tubing was folded, which halved the magnification factor of the sweller[1] but doubled its working range.[2]

For external application, the length of the transducer was made to match the circumference of the fruits to be tested and attached to a threefold length of adhesive tape. The two were wound around the fruit as shown in fig. 24. A close-fitting belt of three layers of glass fibers embedded in the tape served as a virtually non-extensible backing of the transducer, so that any swelling of the tube would induce a circumferential compression of the test fruit.

The extensibility of the latter type of transducer was tested with a non-com-

[1] Ratio of the linear displacements of (i) the liquid level in the capillary tube and (ii) the opposite faces of the sweller.
[2] Greatest possible displacement of the opposite faces between which the sweller is placed.

pressible body, a glass jar of 60 mm diameter. The hydraulic sweller and its capillary connecting tube were filled with mercury to induce variable pressure. The column height was gradually increased to 50 cm, starting at 10 cm. This caused some mercury to flow into the hydraulic sweller, equivalent to a 0.06 mm increase of its thickness. Upon returning to 10 cm Hg, 0.012 mm remained as permanent deformation. Repeating the cycle resulted in an intake, equivalent to 0.05 mm increase of thickness at 50 cm Hg and a permanent deformation of another 0.004 mm added to the previous one. Apparently such irreversible deformations can be minimized by pre-stretching the belt. To that end, before using a belt in an experiment on a fruit, I applied on it a pressure of two atmospheres during a few seconds.

I have used hydraulic swellers either externally – the hydraulic belt – or internally, to evaluate softening rates of tomatoes, apples and pears (see section 5.3.).

Four techniques were applied:

I. The capillary tube of the sweller was fixed so that a vertical column of manometer liquid (kerosene) of one meter height created a hydrostatic pressure in the sweller. After the system had been allowed one hour to settle, the position of the meniscus was marked on the outside of the capillary and the mark was pulled up to the one-meter level. The position of the meniscus was marked again on successive days and the marks were pulled up daily to the one-meter level. The position of the resulting series of marks, recorded against time, indicated the rate of deformation of the sweller.

With this technique, a few internal tests have been made with apples and pears placed in a very humid atmosphere at 23 °C. As a reference (zero deformation indicator) another hydraulic sweller was embedded in a block of WOOD's metal. As compared with this reference, the fruit showed an asymptotic fall that after a few days changed into a rapid downward movement. This sudden weakening apparently resulted from decay, which was found afterwards in the compressed area of the test fruits.

II. This set-up differed from I in that a small funnel, made from 5 mm bore tubing, was connected to the open (upper) end of the capillary and the system including the funnel was filled with manometer liquid. After the sweller had been put into place, the funnel was fixed at a suitable level above the sweller, creating a hydrostatic pressure. The resulting inflow of liquid into the sweller scarcely lowered the meniscus in the funnel, so the hydrostatic pressure was kept virtually constant. The downward movement of the liquid was marked with a small air bubble introduced into the capillary with the aid of a hypodermic syringe and the lower meniscus of the bubble was read at suitable intervals of time. The indicator bubble had to be renewed every few days as it gradually dissolved in the liquid.

III. The sweller was provided with three meters of capillary tubing. The open end of this tube with two meters length was fixed on a table top. The remaining part allowed the sweller with the test fruit to be moved up and down. In that way, hydrostatic pressures of e.g. 10 and 70 cm kerosene were created alternately at a rate of one cycle a day. The successive positions of the kerosene meniscus (or of an indicator bubble) in the horizontal part of the capillary were read and recorded against time. In that way the rates of deformation at 10 cm pressure and at 70 cm pressure could be compared.

IV. This set-up differed from III in that the open ends of several capillaries that had been fixed parallel on a table top were connected to a manifold which was subjected to different air pressures, with the aid of one or two air-in-water overflow systems. This system was more elaborate but also more versatile than III, and could be operated without moving the test objects.

With techniques III and IV meniscus displacements over a distance of two meters could be read conveniently. The use of compressive belts sometimes necessitated still greater distances to be read. Instead of using a greater length of horizontal manometer tubing, the meniscus was made to return after it had travelled about two meters. To that end, a second capillary had been connected to the belt; by way of this additional capillary, some more liquid was introduced into the system, with a hypodermic syringe. After that the series of readings could be continued, the additional capillary being closed with a pin inserted into its open end.

5.2.5. *Additional provisions and remarks*

5.2.5.1. Sand support, three-balls support

The sand support referred to in section 5.1.14 consisted of a glass tumbler, 8 cm high, filled with fine dry sand. Three very small metal feet being cemented to its bottom prevented spilled grains of sand to be crushed or to slip away under the tumbler, and thus to interfere with compression readings.

To prepare a sand support the tumbler was half filled, the test fruit was placed on the sand surface, and more sand was added until the test fruit was at least half immersed. Then, the tumbler bottom was gently knocked against a hard surface, in order to pack the sand more tightly. With a little practice, the top of the test fruit which was to provide the area of contact with the plunger of the compressor, could be brought up to the exact height which was indicated by two marks cemented to the brim of the tumbler.

An objection to the use of a sand support might be that the test fruit, when subjected to a heavy load, would be pressed down into the sand and thus simulate an irreversible compression.

This possibility was tested with the METTLER P-10 Compressor on two dummies, a sphere of hard wood, diameter 68 mm, and a hollow rubber ball, diameter 64 mm. I loaded each repeatedly with 5 kg and renewed the sand support after each series of five loading-unloading cycles.

On the wooden ball the effect of loading was a downward movement of the plunger, which came to a standstill when the balance scale had shifted 60 grams on the average, the range of shifts being between 42 and 63 grams. By then the load on the ball was apparently $5000 - 60 = 4940$ g and the depth of compression was $60/1322 = 0.045$ mm on the average.

On the rubber ball the effect of loading with 5 kg was more complicated because of the high elasticity of the object. Initially the plunger came down

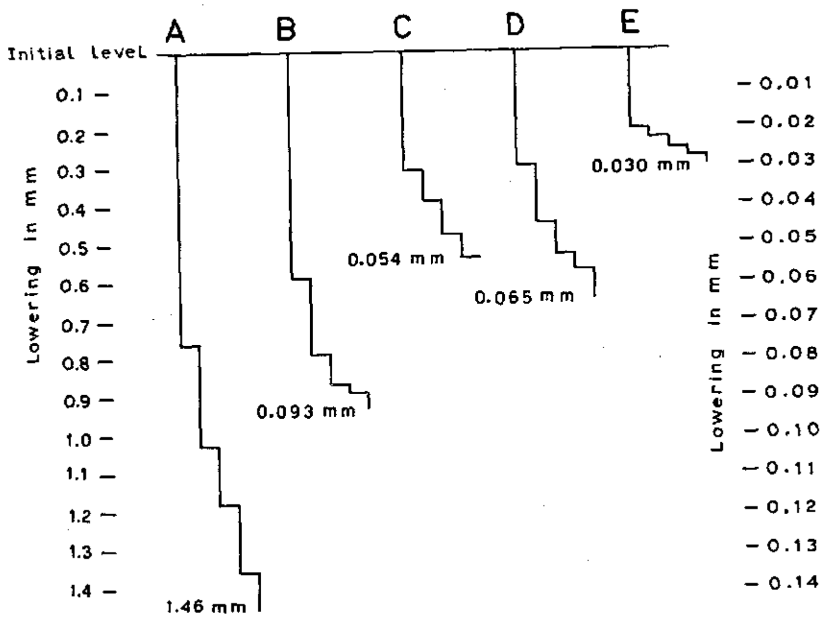


FIG. 25. Step by step sinking induced by repeated loading-unloading cycles. Test objects: A - rubber ball, lying on sand; B - rubber ball, partly immersed in sand; C - rubber ball, partly immersed, sand rammed down; D - rubber ball, partly immersed, sand rammed down and moistened with water; E -wooden ball, partly immersed, sand rammed down. Scale magnification of A is one tenth the other scales.

until the scale shift was 4210 grams, then it bounced back a certain distance after which it came down again moving ever more slowly. As this creeping deformation unduly retarded the determinations, I removed the 5 kg load from the balance pan as soon as the initial compression reversed. By then the load on the ball was 5000 – 4210 = 790 g and the depth of compression was 4210/1322 = 3.2 mm.

In this way the performance of a sand support was tested with a very heavy load as well as with a very flexible test object.

Fig. 25 shows that repeated loading-unloading cycles cause a test object to sink ever deeper into a sand support. These irreversible displacements are greatest when the sand has not been rammed down as in A and B. In all series of five compressions, the first loading caused by far the greatest displacement: 0.02 to 0.03 mm under the most favourable conditions. This type of lowering was repeatedly found when apples, in a well-prepared sand support, were loaded with about 100 grams. It can be mistaken for the yield point which however occurs at higher loads and which leads to much greater displacements.

After having been rammed down a sand support does not seem to be improved by making it more cohesive with a little water (compare C and D).

If the preparation of a sand support is considered too time-consuming, an alternative is a triangle formed by three balls. A test fruit, however oddly shaped, is fixed in any position when laid on this type of support. I used table tennis balls cemented together with 'araldite' resin.

5.2.5.2. Artificially induced firmness changes

Circumstances prevented the methods described previously from being tested extensively on fruits that were actually softening during a long stay in the respirotron. As a substitute for aging, test fruits were subjected to an injurious electric current ('electro-lesion'). This brought about a rapid change in firmness as measured by all methods so far tested, including resonance tests.

A pair of blade- shaped electrodes were inserted into the tissue, facing each other and with three quarters of the diameter of the fruit between them. The set-up was allowed to rest on its support for about an hour, after which a first series of firmness tests was carried out. This first series was continued until it satisfied requirements of consistency. Then, other conditions being left unchanged an alternating current, 50 mA 50 Hz, was sent through the electrodes during a minute. A second series of firmness tests was made immediately after that.

In apples the electric treatment resulted in infiltration of the intercellular space between the electrodes; the affected tissue turned brown within a day or two; the tissue not between the electrodes was apparently sound.

As another substratum that changed its rheological properties a jellifying mixture was used that was prepared by rapidly mixing equal volumes of 0.3 n sodium silicate and 0.3 n hydrochloric acid, both coloured with bromothymol blue. The neutral liquid was transferred to a glass tube, 15 cm long, 7 mm internal diameter and closed at one end. A 'hydraulic sweller' of 12 cm length was immersed into the mixture. The air pressure at the open end of the capillary of the sweller was made to alternate between –26.5 and +21 cm H_2O with the aid of a double air-in-water overflow system and a three-way stopcock. As shown in fig. 26, the oscillations of the liquid level in the capillary tube changed their amplitude from 207 to 31 units during solidification of the mixture. When water was used instead of the jellifying mixture, the amplitude remained unchanged.

The change occurring also affected the *shape* of the oscillations: the time-displacement curves were straight when pertaining to a viscous liquid and about logarithmic in case of the elastic gel.

5.2.5.3. Shrinkage of the test object.

Although the hydraulic sweller when subjected to alternating air pressures would clearly reveal a change in the rheological properties of the test object, it might be affected similarly by shrinkage of the object. This assumption was tested on a virtually incompressible body, a glass jar of 64 mm diameter supplied with a compressive belt. The belt was subjected to air pressure alternating between 10 and 200 cm H_2O, with the aid of a double air-in-water overflow system and a three-way stopcock, which was reversed every 100 seconds. As shown in fig. 27, the amplitude of the oscillating liquid level in the capillary tube of the transducer was small if the belt was wound tightly around the test

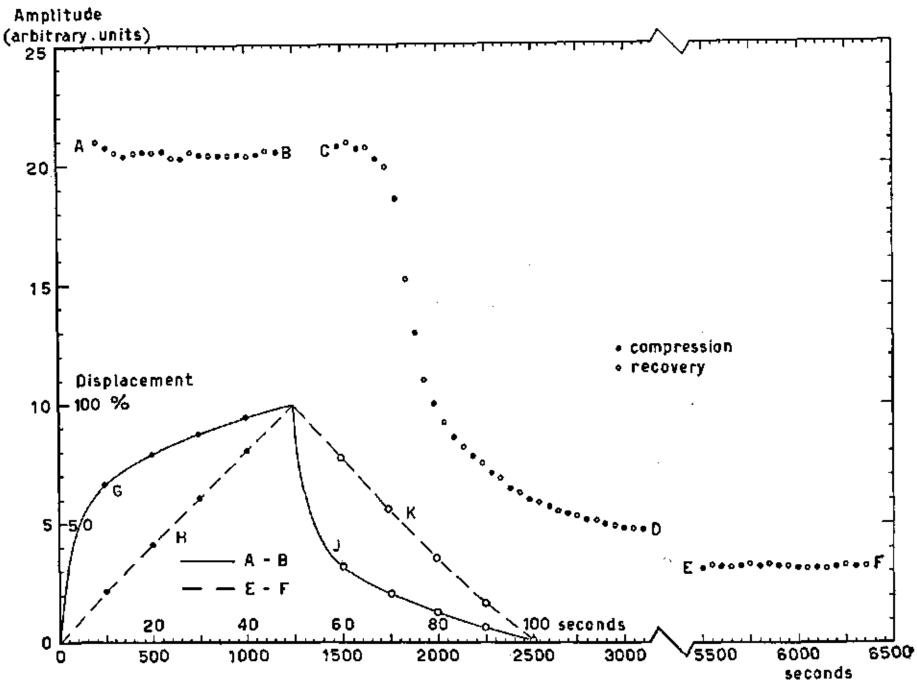


FIG. 26. Amplitudes induced by successive loading-unloading cycles (2 × 50 seconds) of a hydraulic sweller in water (A-B), in a jellifying mixture (C-D), and in solid silica jelly (E-F). Time-displacement curves (in % of the 50-seconds amplitude) in solid jelly (G and J) and in either water or liquid silica mixture (H and K).

object, but was much larger if the belt was wound less tightly; the loosening of the belt evidently had the same effect a sudden softening of the test object would have.

The loosening resulted in 0.05 cc additional intake of manometer liquid into the hydraulic sweller; as the area of contact of the sweller with the test object was 6 × 120 mm^2 and as it just covered the circumference of the test object, the intake of liquid was equivalent to 0.14 mm 'apparent shrinkage'.

The fall of the curve C-D in fig. 27 indicates that the belt contracted slowly after having been loosened. Fig. 27 also shows that loosening and tightening the belt barely affected the *shape* of the curve, contrarily to the effect of the rheological change discussed in section 5.2.5.2. Apparently the amplitude and the rate of displacement are not interdependent and are to be considered separately, therefore.

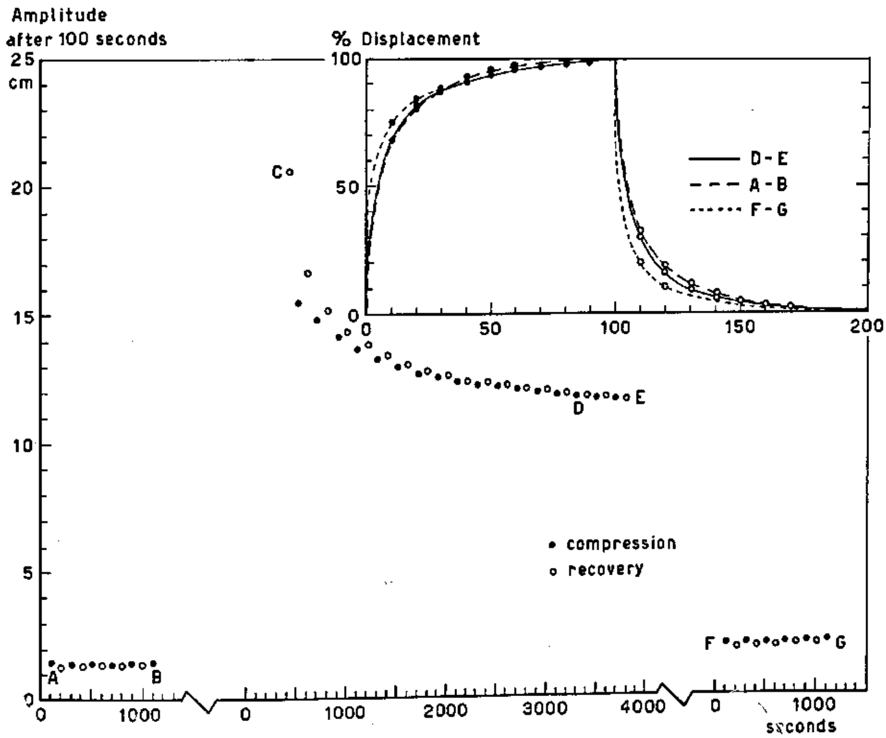


FIG. 27. Performance of compressive belt during simulated shrinkage of a non-compressible test object. Successive amplitudes of oscillating manometer liquid when the belt is wound tightly (A-B), when loosened (C-D-E) and when tightened again (F-G). Time displacement characteristics of three types of oscillations are shown in the insert.

5.3. RESULTS

With the three types of apparatus described in section 5.2. determinations concerning firmness, softening and yield point were made on tomatoes and on apples.

5.3.1. *Suppression of creeping deformation*

As discussed in section 5.1.6 the rate of non-destructive compression at constant load will slow down until it reaches an equilibrium that cannot be distinguished from the normal rate of shrinkage of the test fruit. This equilibrium may be assumed to be reached sooner at decreasing load than at constant load. This assumption was tested by comparing the performance of METTLER P-10 and P-120 compressors. In these apparatus, one millimeter downward movement of the plunger reduced the load on the test fruit by respectively 1322 gram and 1.878 gram.

In a series of compression tests on several areas of the equator of tomatoes

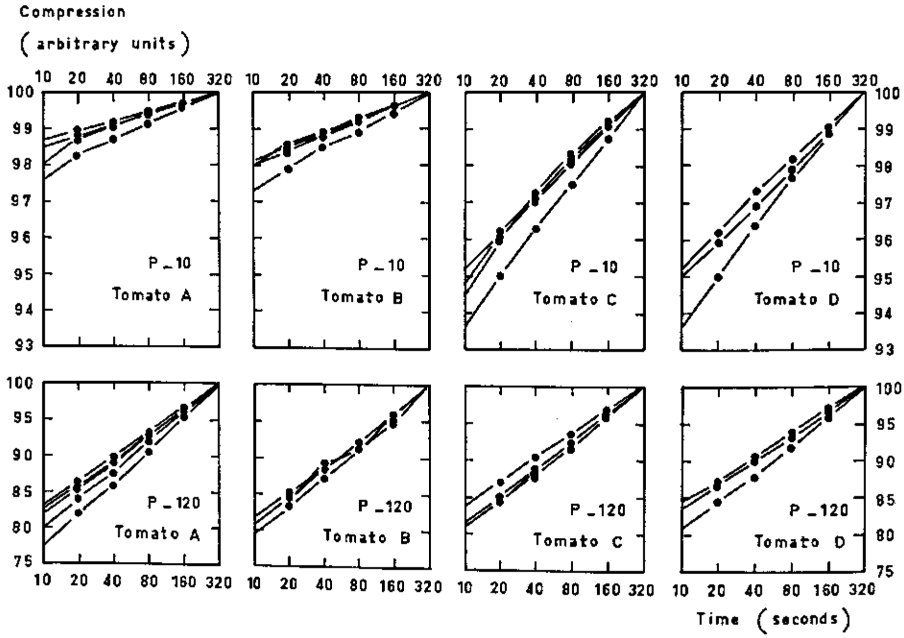


FIG. 28. Time-deformation curves of tomato fruits (A and B: fully ripe; C and D: in early stage of ripening), when compressed with METTLER Compressor P-10 or P-120. In tomato A, ten areas on the equator of the fruit have been compressed, five with P-10, the alternating five with P-120; in the fruits B, C and D respectively 8, 8 and 6 areas were compressed. Compression depths were read after 10, 20, 40, 80, 160 and 320 seconds load, the depth at 320 seconds was taken as unit (100%). Scale of ordinates "P-10" is five times as wide as that for "P-120".

(see fig. 28), the P-10 METTLER compressor was weighted with 3 kg, the weight was removed after 320 seconds. At any moment during the compression the load on the tests fruit was assumed to be the weight on the balance pan reduced by the shift of the balance scale. In alternate tests with the METTLER P-120 Compressor the balance pan was loaded with a weight that was about equal to the load attained after 320 seconds (to be called L. 320) in the preceding test with P-10. The average value of L. 320 was found to be 410 gram with P-10, and 409 gram with P-120; the average reduction of the load during 320 seconds compression was 2590 gram with P-10, and was 2 to 5 gram with P-120. Although equal loads had been attained in both ways, P-120 had provided a virtually constant load as compared to P-10.

In both systems, deformation was rapid at the start and slackened strongly. In fig. 28 series of readings have been plotted against time. Deformation at 320 seconds was taken as 100%, most of which occurred within 10 seconds. In the test with P-120 the fraction of the deformation occurring *after* 10 seconds was 19.1% in soft fruits, and 17.7% in unripe fruits; the difference was statistically unsignificant. In the tests with P-10 the fraction of the deformation occurring after 10 seconds was no more than 1.9% in the soft fruits, and 5.4% in the unripe fruits; this difference was highly significant.

Apparently, firmness testing with P-10 as compared to P-120 can be done more rapidly and the results are more discriminating.

The results of compression tests with P-10 shown in fig. 28 allow the two ripe tomatoes to be clearly distinguished from the two fruits turning red. The first reading of the first series of compressions made on each of the four fruits showed a balance scale shift of 2718 g and 2760 g in tomatoes A and B, and 2225 g and 2202 g in tomatoes C and D. Most probably, a single reading made after 10 seconds compression with P-10 will result in a firmness value", which is sufficiently exact to characterize a tomato fruit.

Nearly all series of tests show straight lines when plotted against the logarithm of time. This appears both at decreasing load (P-10) and at constant load (as assumed with P-120); it has also been observed with K-5-T, a medium range METTLER Compressor that was mentioned in section 5.2.2.

Apparently, the effects of counterpoise and of the resilience of the test fruit are similar.

5.3.2. *Yield point of apple.*

As shown in fig. 29 the yield point can be determined in different areas of a single apple with the aid of a METTLER Compressor and a sand support. The fruit was embedded in a different position after each test.

The three force-compression curves are slightly bent near their origin; most probably this is due to sagging of the sand support as I found repeatedly with other apples that were loaded up to 100 grams. In the example given, the weight

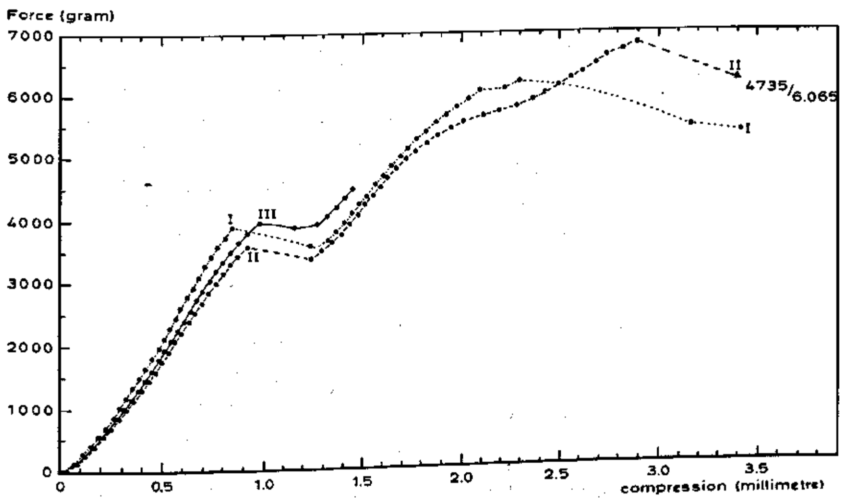


FIG. 29. Force-compression relationships of three areas on the equator of a Golden Delicious apple (I on blush side, II and III on green side of fruit), as determined with METTLER P-10 compressor. The yield point appears at about 0.9 mm, skin rupture at 2.3 to 2.9 mm.

on the balance pan had been increased by 200 grams every 40 seconds; the yield point appeared after the 24th (II) or the 25th (I and III) addition when compression was about 1 mm.

Breakdown of the fruit did not occur until compression was 2 to 3 mm.

Yielding of the apple flesh led to a sudden decrease of the load on the plunger because of the counterpoise in the METTLER balance. A few more additons to the weight on the balance pan restored the load and made the plunger sink another 0.55 mm (I), 0.41 mm (II), and 0.265 mm (III).

Unlike the compression leading to breakdown which is commonly known as the *penetrometer value*, the load at the yield point and the compression immediately preceding yield appear to be highly consistent and definite.

5.3.3. *Yield point of tomato.*

I tested the possible existence of a yield point in tomato on fruits that were supposed to have not been bruised before. Fruits of the Moneymaker variety were handled in such a way that part of their surface was not touched before they were subjected to a compression test. To that purpose they were held at their stalk when picked in a nearby glasshouse, and while being placed in a sand support; they were carried from the glasshouse to the compressor embedded in cotton wool.

As may be concluded from fig. 30 a steadily progressing compression did not reveal a yield point in unripe tomatoes, although it resulted in an indentation left in the surface of the test fruit.

Another way to find a possible yield point in tomato is by studying the recovery of the fruit after loading and unloading. Recovery will be incomplete as soon as a yield point has been passed.

The following test was made with a soft-ripe tomato as a test fruit, and with the P-120 balance as a compressor. The balance pan was loaded successively with 10 g, 20 g, 30 g, etc, and the weight was removed after one minute of compression. Immediately after the weight had been removed, the plunger was lowered with the aid of the tare mechanism of the balance, until it just touched the fruit again. During recovery the fruit lifted the plunger, causing the balance scale to shift. After one minute recovery the scale was read and the balance pan was loaded again, either with the same weight (to repeat a loading-unloading cycle) or with ten grams more.

The lift of the plunger caused the counterweight of the balance to increase 1.878 g/mm, which is considered a negligible counteraction.

In fig. 31 the successive positions of the plunger after one minute recovery are summarized. Evidently, recovery was not complete within one minute, even after loading and unloading only 10 g. Throughout the series of loading-unloading cycles recovery amounted to about 40% of the preceding compression. This is demonstrated in fig. 31, two selected data being added: where compression had been 1.25 mm recovery was 0.52 mm and where compression had been 2.4 mm recovery was 1.04 mm. The 40% ratio occurred up to the highest load without a break, i.e., without passing a level that might be con-

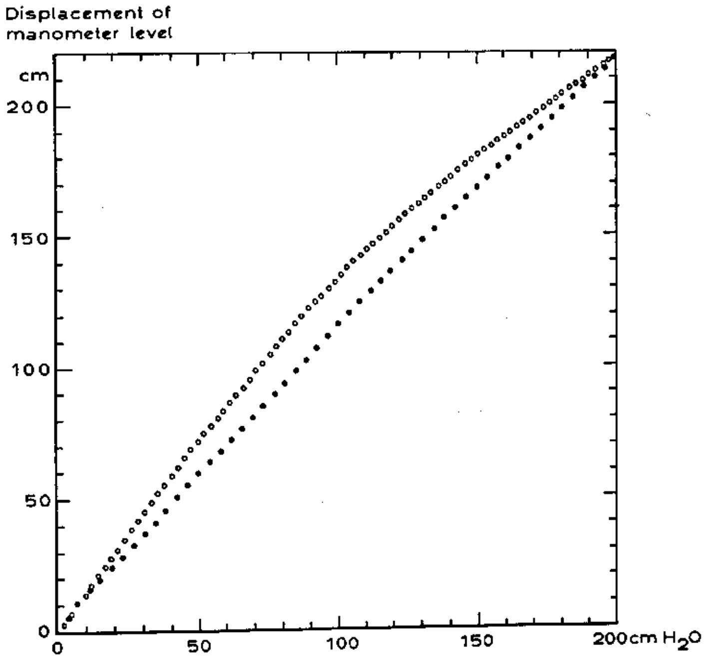


FIG. 30. Time-deformation curves of two tomato fruits, both in an early stage of ripening, as determined with the bellows apparatus subjected to a steadily increasing pressure. Dots and circles represent readings of displacement taken every 5 seconds. The division of the abscissa is based upon the assumption that the coinciding increments of pressure have been linear up to the ultimate pressure of 200 cm H_2O. The coincidence of the ultimate displacement values may be incidental; it represents 7 mm compression of the test fruits. Within that range, no yield point had been attained, as no break appears in the curves.

sidered a yield point. Nevertheless, the step by step compression resulted in a definite indentation left in the surface of the test fruit.

5.3.4. *Compression over cross-wall and over locule.*

From tomato compression data reported by HAMSON (1952) one may conclude that the surface areas over cross-walls appear 4% firmer than those over locules. Still, the data reported are extremely variable; consistent data might be obtained with more appropriate material and methods. As such, tomato fruits with a single median cross-wall were used and compressed with the bellows apparatus (standard method), while embedded in a sand support (see fig. 32).

The position of the cross-wall was estimated from the shape of the blossom-end scar, and verified after the test by cutting the fruits transversely. Mismatch of the two was found in 10 fruits, the test results of which have been discarded. The test results of the remaining 15 fruits are presented in fig. 32. For each specimen, blossom-end firmness is plotted against four other firmness values which have been determined on the equator of the fruit at its intersections with the median of the cross-wall and with the medians of the two locules.

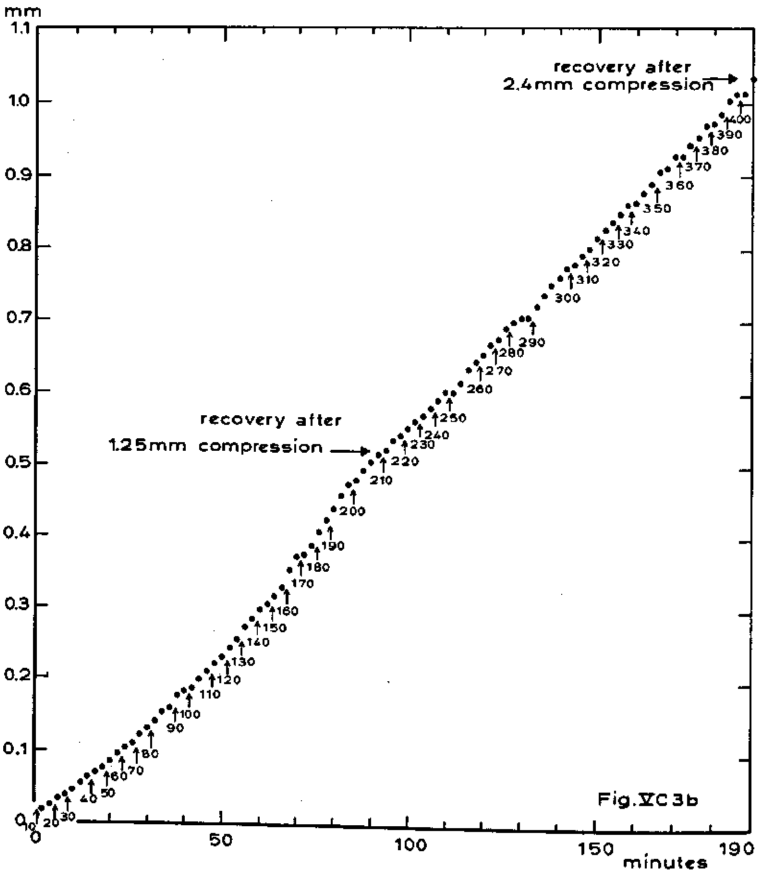


FIG. 31. Incomplete recovery of a tomato fruit after loading and unloading METTLER P-120 compressor. Each dot represents position of plunger 60 seconds after unloading. Vertical arrows indicate increase of load. Numbers refer to weight in grams on the balance pan. Because of incomplete recovery after repeated loading the plunger has lowered one millimeter in three hours. The rate of lowering is virtually uniform, indicating that no definite yield point has been surpassed although the fruit was compressed 2.4 mm and showed a distinct indentation after the test.

The five data show differences and interrelations that may be summarized as follows:

- firmness values over cross-walls are higher than those over locules, the difference amounting to 4.7% ± 1.16;
- correlation between blossom-end firmness and cross-wall firmness is 0.89 (P = 0.99);
- correlation between blossom-end firmness and locule firmness is 0.88 (P = 0.99);
- rank correlation[1] between blossom-end firmness and an arbitrary evalua-

[1] Rank correlation is calculated in case some variable is compared with an arbitrary evaluation, such as the numeric notation of tomato colours.

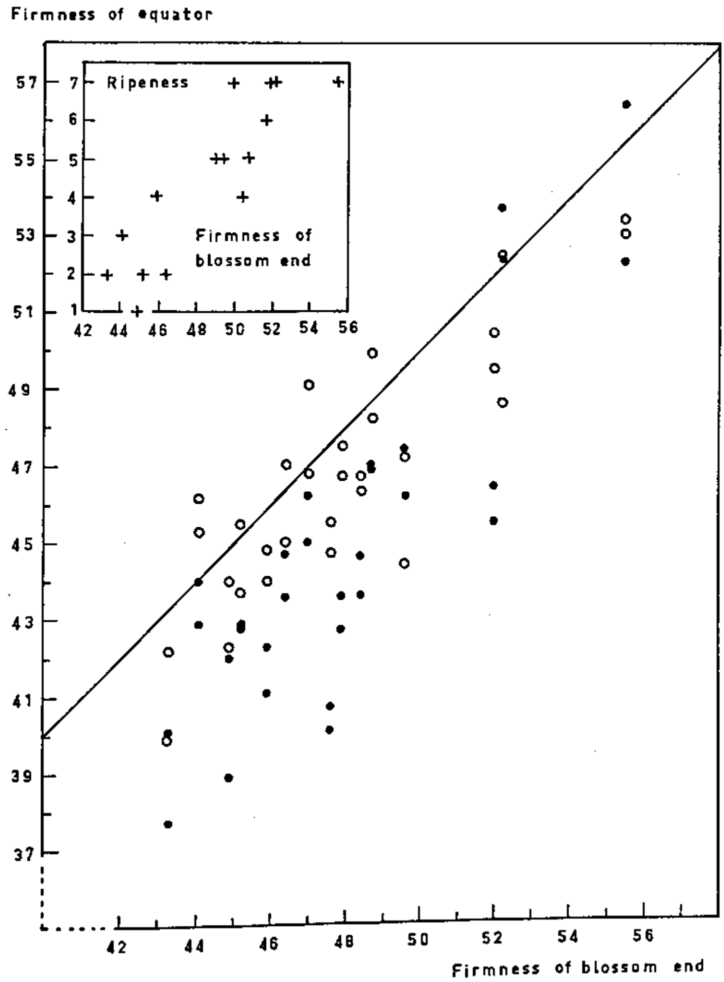


FIG. 32. Firmness value of blossom end of 15 tomato fruits, as related to firmness values of their equator (diagonal line represents equality), and as related to rank number of ripeness (insert). Determination have been made on the equator at its intersections with (●) median of locule, or with (○) median of cross-wall. Rank numbers of ripeness: 7 - light green; 6 - pale green; 5 - pale orange; 4 - orange; 3 - red; 2 - ripe; 1 - overripe.

tion of ripeness (see fig. 32. insert) is 0.89 (P = 0.95).

Preliminary conclusions from these observations are:

- a single firmness value obtained from a restricted area of the tomato's equator is not representative; it may be 'too high' when from a cross-wall, it may be 'too low' when from a locule;
- equator firmness values are satisfactorily correlated with blossom-end firmness values so that the latter appear to represent 'over-all firmness' of a tomato fruit;

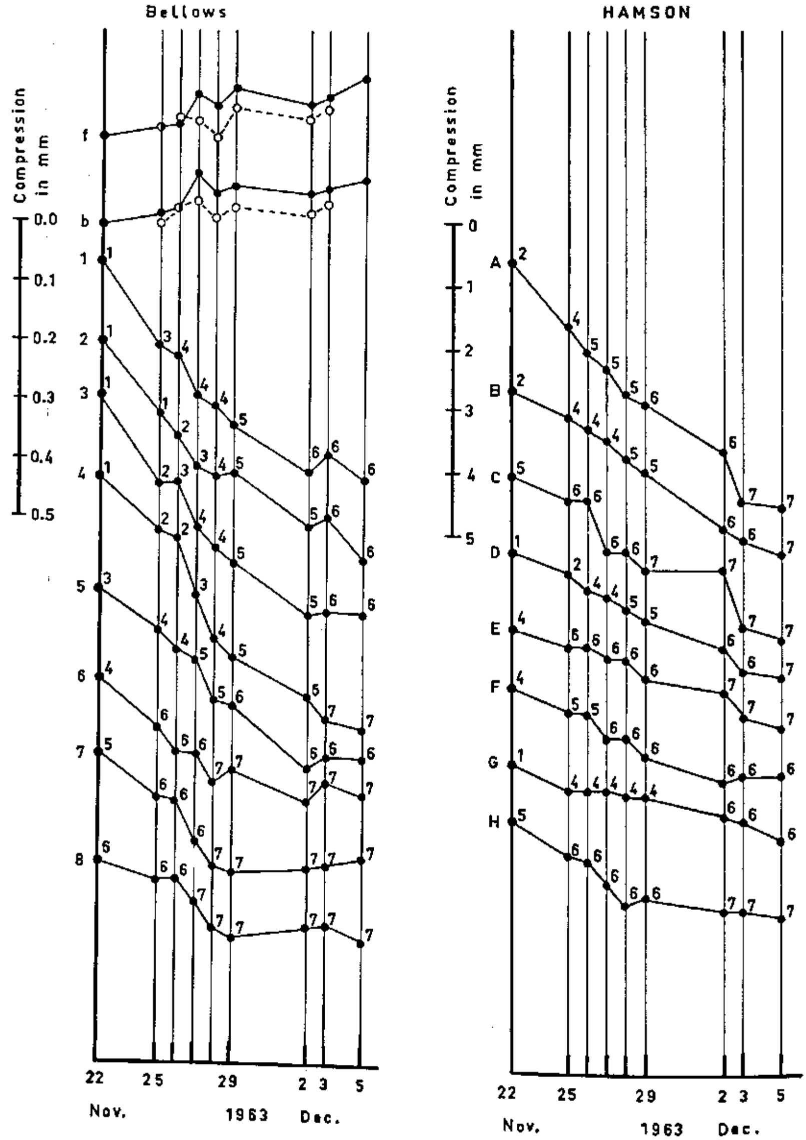


FIG. 33. Decline of blossom-end firmness of tomato in the course of 14 days at 10°C. Compressibility of 8 fruits (1-8) was measured with Bellows method, of another 8 fruits (A-H) with HAMSON method, slightly modified. "Free extension" (f) and "blocked extension" (b) were determined concurrently, both before (solid lines) and after (broken lines) a series of compression readings was made. At each compression the stage of ripening of the test fruit (see table 1) is noted. A ten-to-one relation between the two scales (compression in millimeters) shows similar types of decline in curves 1 and A.

- changes of blossom-end firmness keep pace with colour shifts in ripening tomatoes. This conclusion is supported by the data discussed below.

5.3.5. *Softening of blossom-end.*

Blossom-end firmness in relation to time (fig. 33) and in relation to colour (table 1) was tested comparatively with the Bellows device and with a modified HAMSON method (0.5 kg load, plunger Ø 10 mm, compression read after 30 seconds, one millimeter downward movement of the plunger causing 5 grams decrease of load). Test fruits were picked, all at the same day, from a few plants that had been left from a breeding experiment. They were in various stages of ripeness when picked and afterwards turned out to contain many cavities. They were divided into two lots, 2 × 23 specimens, of apparently similar composition. Both were stored at 18 °C in wooden trays covered with polyethylene film. Each blossom end was tested almost daily, the test dates being shown in fig. 33, the test fruit resting on a three-balls support. A series of 23 tests took one hour and a half with the HAMSON method, two and a half hours with the Bellows method.

Fig. 33 shows a few typical trends selected from the two sets of twenty-three curves.

Both 'free extension' and 'blocked extension' show a gradual rise in the course of 14 days, the cause of which is not known. If this zero shift happened in the fruit firmness readings as well, the softening of the fruits would actually run somewhat steeper than shown in the figure.

Both 'free extension' and 'blocked extension' are affected by a series of 23 compressions with the bellows. The apparent increase is another type of zero

TABLE 1: Blossom-end firmness in relation to ripeness of 2 × 23 specimens of tomato fruit, each tested 9 times

Column A: rank number and definition of ripeness stage
„ B to D: compressions made with HAMSON method
„ E to G: compressions made with Bellows method
„ B and E: number of readings taken
„ C and F: average compression depth, in millimeters
„ D and G: variation coefficient of the compression depths

	HAMSON method			Bellows method		
A	B	C	D	E	F	G
1 green	9	1.09	18%	9	0.82	7%
2 pale green	12	1.41	15%	16	0.865	5%
3 slightly turning	7	1.54	15%	13	0.92	6%
4 turning	29	1.78	24%	31	1.00	6%
5 orange	37	2.32	20%	31	1.07	7%
6 red, orange red	81	2.86	20%	60	1.12	5.5%
7 dark red	32	4.00	16.5%	47	1.22	3.5%

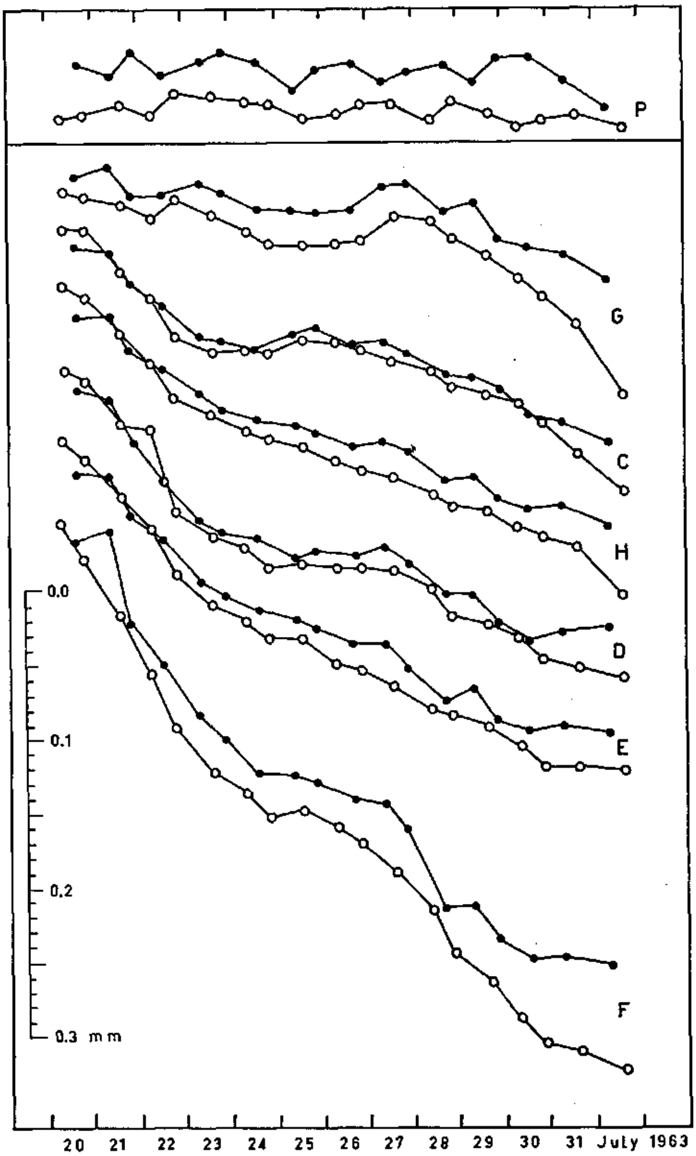


FIG. 34. Performance of hydraulic belts on tomato fruits (C to H) and on an incompressible body (P). Belts were loaded alternately with pressure of 16 cm (●——●) and of 61 cm (○——○) kerosene column. C to H: corrected data, i.e. readings reduced by concurrent data from the incompressible body P. The scale of 0.0 to 0.3 mm represents a supposed plane-parallel dilatation of the swellers in the belts as calculated from the volume of liquid taken in. The apparent decrease of the diameter of the constricted area is twice the fall of the curves. This fall, as well as the divergence of high-pressure curves from low-pressure curves, is due to both softening and shrinkage.

Specimen	Colour		Consistency
	at the start	at end of test	at end of test
C	green	orange	hard
D	green	orange-red	firm
E	green	orange-red	firm
F	turning	orange-red	firm to soft
G	red	red	soft
H	orange	red	firm

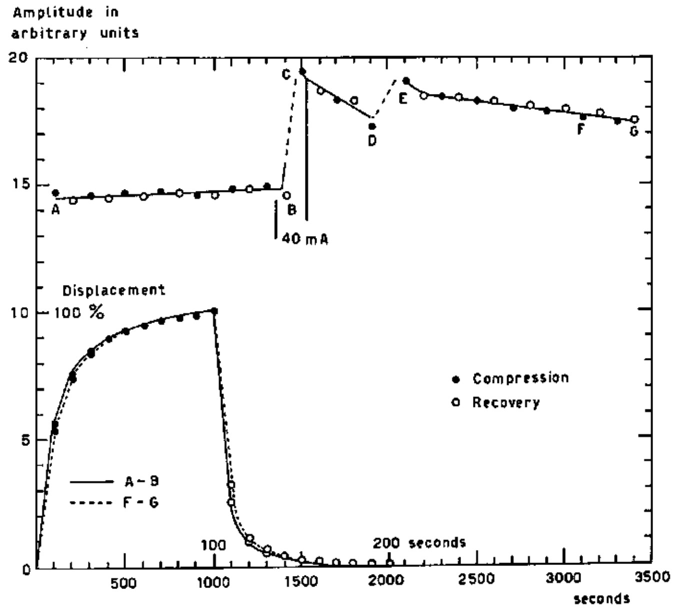


FIG. 35. Firmness of an apple fruit (Ingrid Marie), as determined with a pulsating hydraulic belt (pressure alternately 10 and 200 cm water) and as affected by an electric current. Amplitude of oscillating liquid increases during the electric treatment (B-C), whereas its time-displacement characteristics are barely affected. Break D-E is caused by a temporary failure of the apparatus.

shift; the resilience of the bellows seems to increase as a result of the manipulations (thixotropy).

The 'Bellows' trends fall most steeply in the first stages of ripeness (indicated 1 to 5) and much less in the stages 6 and 7. In the 'HAMSON' trends such a tendency is less conspicuous. Still, in neither of the two sets of trends the relation between 'firmness' and 'ripeness' is as clear as in table 1, p. 63. Here the 'Bellows' data show the smaller variation coefficients, indicating that they are interrelated with 'colour' more closely than the 'HAMSON' data.

5.3.6. *Softening and shrinkage.*

Results obtained with hydraulic belts are known to be affected by softening and by shrinkage of the test fruits (see section 5.2.5.3). The combined effects result in a gradual change as shown in fig. 34. The test fruits were tomatoes, all of the same cultivar, the same provenance and the same picking date, but of different colours when picked. The specimens, each with a hydraulic belt, were kept in the upper part of a large desiccator; in the bottom part a heater and a contact thermometer were immersed in water that was kept at 24.8 ± 0.2 °C and that was stirred with air bubbling through at a rate of 0.1 liter/min. The desiccator was wrapped in a folded blanket and provided a warm and very humid atmosphere. Between the tomatoes, a glass jar, also with a hydraulic belt,

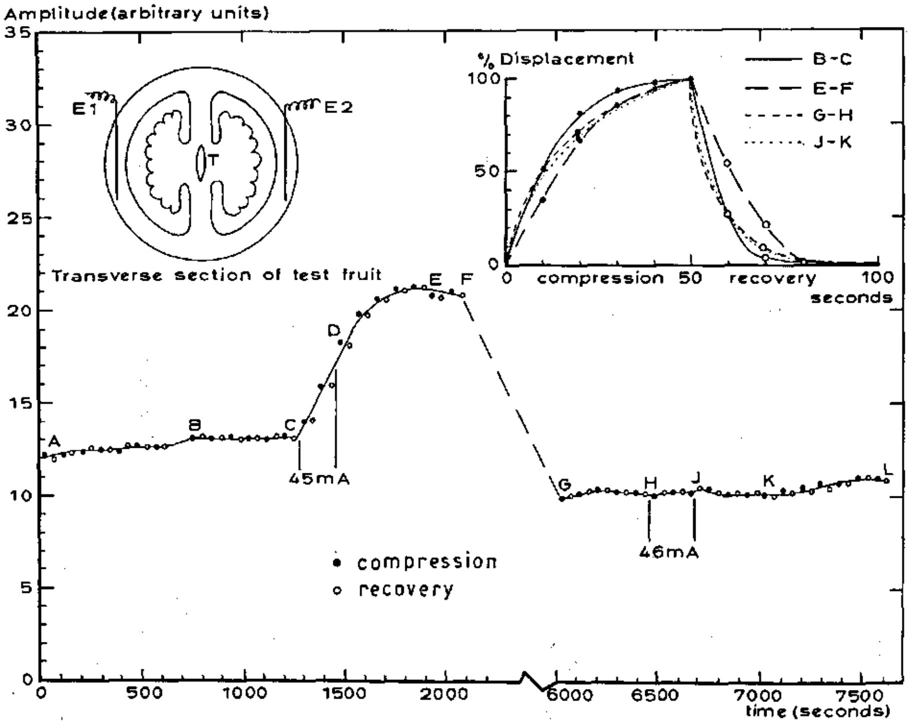


FIG. 36. Firmness of a green tomato fruit, as determined with a pulsating hydraulic sweller (pressure alternately − 25 cm and + 20 cm water), and as affected by an electric current administered repeatedly. First treatment causes an increase (C-D) of amplitude of oscillating liquid, and some straightening of its time-displacement curve (compare B-C and E-F). During the interval F-G 46 milliamperes were administerd twice for a period of 5 minutes. After that, the amplitude had reduced. and time-displacement curves were less straight (compare E-F and G-H). A fourth treatment affected neither the amplitude (H-J) nor the time-displacements characteristics (compare G-H and J-K). Position of electrodes E_1 and E_2 and of sweller T co-axial with test fruit is shown in transverse section of specimen.

served as a non-compressible reference object. The hydraulic belts of all test objects were loaded simultaneously with either 16 or 61 cm kerosene column pressure (by means of technique III, mentioned in section 5.2.4. The position of the meniscus in each capillary was read just before the load was changed. In that way comparable data on trends occurring in the reference body and in the test fruits were obtained.

The data pertaining to the reference body are not due to compression of the test object, but to dilation of the belt for other reasons. A similar tangential and outward dilation would have occurred in the belts around the test fruits; apart from this though, the fruits are also subject to shrinkage and softening during the determinations. The combined effect of shrinkage and softening of the fruits appears after the data have been corrected with the concurrent data of

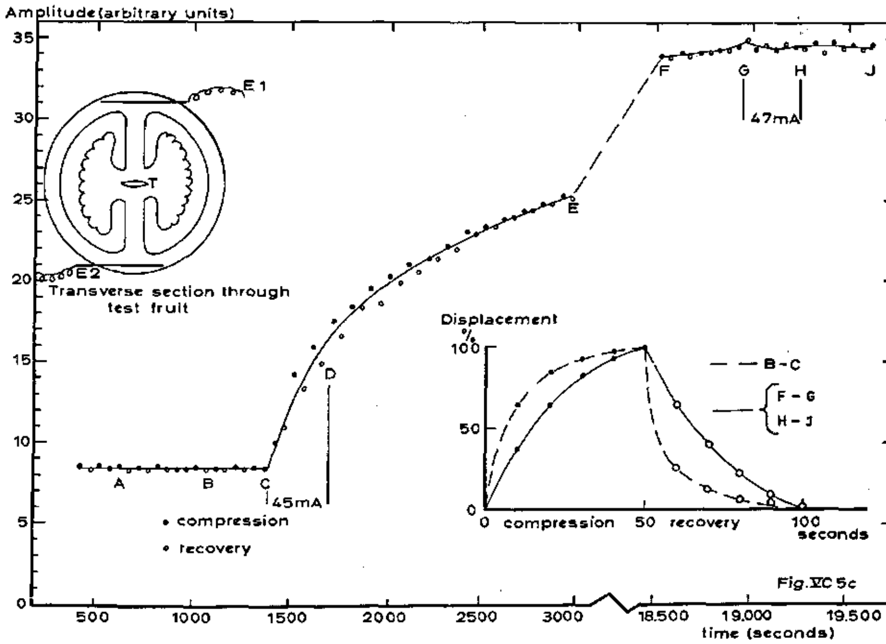


FIG. 37. Firmness of a pink tomato fruit, as determined with pulsating hydraulic sweller (pressure alternately 20 and 100 cm water) and as affected by electric current administered twice. First treatment causes increase (C-D) of amplitude of oscillating liquid, and straightening of its time-displacement curve (compare B-C and F-G). Second treatment affects neither the amplitude nor the time-displacement curve (compare F-G and H-J). Position of electrodes E_1 and E_2 and of sweller T co-axial with test fruit is shown in transverse section of specimen.

the reference object. As the data represent the calculated plane-parallel dilation of the sweller in the belts, and as the sweller in each of the belts spans the test fruit almost completely, the decrease of the test fruit diameter is twice the decline of the curves.

Apparently the combined effect of softening and shrinkage amounts to 0.01 to 0.07 mm/day decrease of the diameter of the fruit. In addition the high-pressure curves tend to diverge from the low-pressure curves in the course of time; this too is to be ascribed to softening and shrinkage. The estimated scatter of the corrected data in the curves is in the order of 0.001 mm.

5.3.7. *Electro-lesion.*

As mentioned in section 5.2.5.2 an electric current will induce a change in the rheological properties of fruits. This change has been found with each method for 'measuring firmness' applied so far. In tomato and apple, the change affected 'hydraulic swellers' as shown in figs. 35 to 39. The oscillation of the liquid in the capillary of the sweller changed to a varying degree.

Fig. 36 and 37 show that a *repetition* of the electric treatment has virtually no

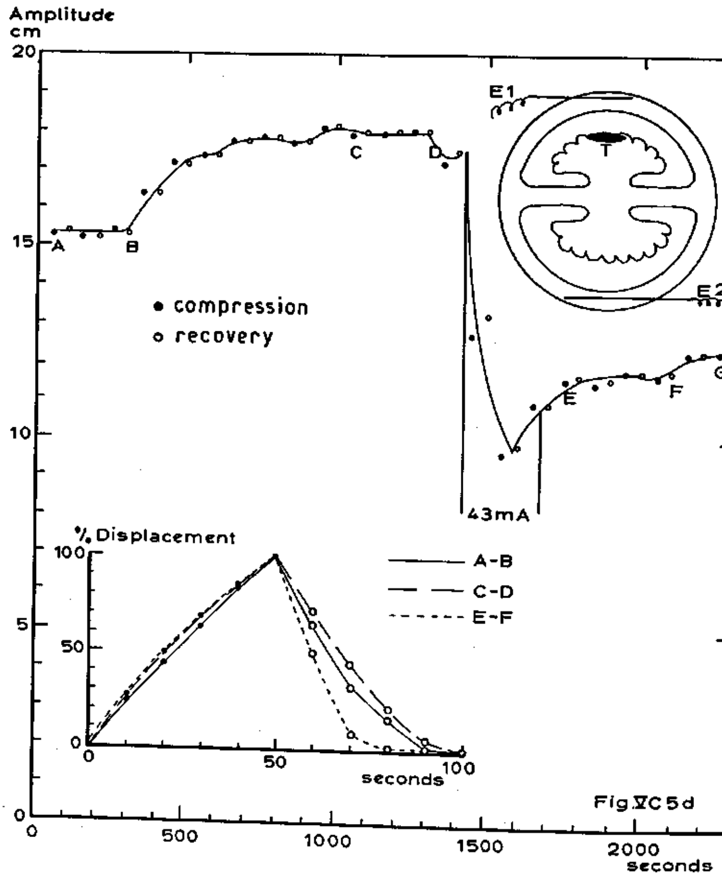


FIG. 38. Firmness of an orange-red tomato fruit, as determined with pulsating hydraulic sweller (pressure alternately −25 cm and +20 cm H_2O) and as affected by an electric current. As compared with figs. 35, 36 and 37, trend of amplitude of oscillating liquid was erratic before current was administered, and was anomalous during and after treatment. Position of electordes E_1 and E_2 and of sweller T (in the pulp, its main axis parallel to the main axis of the fruit) is shown in transverse section of specimen. Semi-liquid nature of the contact area of the sweller causes time-displacement curves to be mainly straight.

effect on the shift of the meniscus; clearly the change after the first treatment indicates the death of the affected tissue.

In most cases the shape of the time-shift curves inserted in the figures indicated that the electric current had an effect *opposite to jellification* (compare 5.2.5 2) After some time, however, the shift would revert; this might be due to an increase of the turgor of the tissue adjacent to the killed part of the fruit by absorption of water released from the killed tissue.

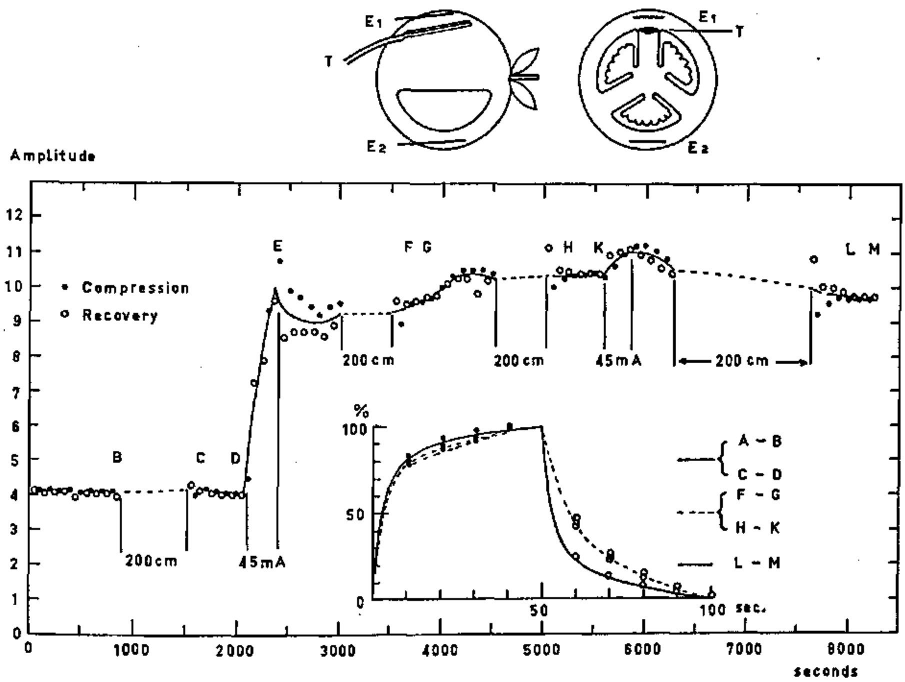


FIG. 39. Firmness of a green tomato, as determined with pulsating hydraulic sweller (pressure alternately 20 cm and 200 cm water), and as affected by (i) electric current administered twice and by (ii) upholding 200 cm pressure during several minutes at a time. After prolonged pressure the trend is unaltered (C and H) or changes in either direction (F and L) apparently without changing the time-displacement characteristics (compare A-B with C-D, and F-G with H-K). First electric treatment (D-E) increases amplitude of oscillating liquid and somewhat straightens its time-displacement curve (compare C-D and E-F), whereas second treatment has minor effect (compare H-K and L-M). Position of electrodes E_1 and E_2 and of sweller T (in a cross-wall, its main axis not parallel with main axis of fruit) is shown in longitudinal and transverse sections of specimen.

6. GENERAL DISCUSSION

INTRODUCTION

The aim of these investigations on physiological functioning was to find its relation to market quality.

As discussed in chapter 1, the appearance of any type of anomaly in the market quality was assumed to be preceded by some deviation from 'normal' functioning. Any knowledge of such deviations was thought to be of interest in the study of storage diseases. Also, the start of a deviation was presumed to pass unnoticed unless special precautions were taken.

Some of these precautions are well known in current investigations on fruit storage physiology, such as the use of test fruits all picked from the same tree, and the provision for thermostated storage conditions. Also, it is common ground that the trends studied, of CO_2 production rates and of softening, are closely connected with the market quality.

On the other hand, the extremely low rate of metabolism in refigerated plant tissue, and the unpredictable moment at which a deviation might start, were thought to necessitate appliances that were not generally adhered to at the time the present study began. As such were regarded the use of *numerous single specimens* (instead of composite samples) as test objects, the provision for *experimental conditions* that should preclude any disturbance of the test objects, and the use of *measuring techniques* of utmost precision.

The probability that a deviation is noticed right after its start increases with the number of specimens that are studied; because of that a feasible number of specimens were studied simultaneously. Possible complications that could have been caused by interactions between specimens were avoided by keeping the specimens separated from each other.

Experimental conditions resembling the essentials of refrigerated storage of fruits and vegetables were thought to include a constant low temperature, constant darkness and an unaltering composition of the atmosphere surrounding the test objects. As an additional factor, the barometric pressure to which the test objects were exposed was kept constant; this was assumed to help reduce the scatter of our data on CO_2 production rate, an assumption that was not actually tested as the manostat system was put to regular use as soon as it was found to work properly.

As to measuring techniques, those adopted were considered to serve the early detection of deviations from normal trends. The limit of early detection was expected to be set by the 'physiological noise level', which is thought to be inherent in fruits and vegetables stored under strictly constant environmental conditions. The physiological noise level should surpass the scatter of data obtained under comparable conditions from a physiologically inert test object.

No such excess was found in our data, both on CO_2 production and on softening; the coefficient of variation of our data was about 1%, both in the live objects and in the standards, i.e. in a flow of test gas serving as a standard for

CO_2 production and in a dummy serving as a standard for softening. Apparently, the scatter of data ought to be further reduced to approach the physiological noise level. But as they are, our results compare favourably with those reported in literature.

Respiration

Methods

The speed and efficiency of our method for measuring CO_2 production rates seem to be a consequence of the use of a highly turbulent absorbing system. MIESSNER (1962) states: 'Die Verwendung eines Gaslifts als Absorber ergibt (...) sehr hohe Wirkungsgrade'. And PERRY *et al* (1963) note that in CO_2 absorption in alkaline solutions 'liquid-phase diffusion is controlling...' and that eddy diffusivities occurring in highly turbulent liquids can be 100,000 times the molecular diffusivities. In our absorber (fig. 6), the very strong turbulence observed seems to be due to a favourable combination of factors. First, the liquid used was less viscous than an aqueous alkaline solution. Also, the gas lift consisted of about equal volumes of gas and liquid, which implied that the gas flow generated by the aspirator that is part of the absorber caused a chain of bubbles and droplets to move at a rate of one meter/sec through the absorber tube. As the volume of circulating liquid was minimal, 2 cm^3, the pH shift caused by absorption of CO_2 occurred with utmost rapidity; this enabled the data to be obtained in rapid succession. Speed would have been impaired by an obstruction of the liquid circulation and/or by an increase of the volume of the absorber, such as would have occurred if pH electrodes had been introduced. Therefore, we had to resort to a pH indicating system without electrodes, such as described with figs. 6 and 7. That system was put to regular use as soon as it was found to work properly .We could not find an opportunity to try out other systems, however promising they might appear.

As to other systems, a gas chromatograph, according to ALEXANDER and FRANCIS (1964) will perform a complete analysis of CO_2, O_2 and N_2 within four minutes. However, much additional work is required to turn the chromatograms into comparable data. On the contrary, our experimental data were produced at a rate of 70 recordings in four hours and allowed comparisons to be made immediately.

In our open-circuit method for measuring CO_2 production rates the free space of the sample containers need not be known. The closed-container method described by SCHIPPERS (1965) implies the residual space of the sample containers to be determined by immersing the test specimens in water.

SCHIPPERS (1965) determined the respiration rate of onion bulbs by measuring the accumulated effect with the aid of a LLOYD-HALDANE volumetric gas analysis apparatus. The precision of the apparatus is 0.02% of the total burette volume, as discussed in the next paragraph. That implies that the accumulated effect of respiration had to be allowed to proceed to a 2% content of CO_2 and/or to a 2% depletion of oxygen in order to have it determined with (0.02% vol):

(2% vol) = 1% variation coefficient. Althought the physiological effect of a 2% content of CO_2 may be negligible in a short-run experiment, it cannot fail to affect the 'normal' respiration and is inadmissible in precise long-term investigations on fruits and vegetables, therefore.

SHEPHARD (1966) studied the results of numerous analyses that had been performed in a routine way on expired air, either with the volumetric LLOYD-HALDANE apparatus or with the BECKMAN paramagnetic oxygen analyser, Type E-2. The LLOYD-HALDANE showed a 'combined reliability of sampling and analysis (...) well within the range ± 0.02%', and the BECKMAN showed 0.05% as a 'standard deviation due to errors'.

YOUNG and BIALE (1962) used a BECKMAN Model C paramagnetic oxygen analyser to determine a decrease of oxygen content due to respiration, and found a precision of 0.02% O_2.

These standard deviations, in the order of 0.02 to 0.05% O_2, are strikingly different from those reported for measurements of CO_2 production rates. VAN OORSCHOT and BELKSMA (1961) used a LISTON-BECKER infrared gas analyser, Model 15 A, and found about one part per million (0.0001 % CO_2) as a standard deviation. We found a standard deviation of about 3 parts per million to exist between data read at intervals of one or more days. These findings support the assumption that very low rates of CO_2 production can be measured far more precisely than the corresponding rates of oxygen consumption.

Results

In the course of our work, individual CO_2 production rates of several hundreds of apple fruits were studied. In many specimens the trends closely followed those reported in literature, and were considered 'normal'. In other specimens, deviations form a 'normal' course occurred, which were considered 'abnormal'.

A deviation was found to be induced by a thermal stimulus or by a mechanical stimulus. Various effects of that kind are discussed in section 4.2.2.3. An interesting effect was a divergation occurring between two identical trends after a thermal stimulus. The two test objects had been subjected to different pretreatments, which otherwise might have shown their effect at a much later date. Apart from the possible diagnostic value, the present study is not primarily interested in reactions induced by operations that are known to affect ripening (LANDFALD, 1961).

The leading idea of this study is that the start of a deviation from 'normal' to 'abnormal' marks a transition in the storage life of a specimen; such a transition will entail other effects; all effects together lead to a change in the market quality of the fruit; the nature of the transition can be specified as soon as all effects are known.

As an example, yielding to fungal attack might be considered a transition in a fruit. One of its most conspicuous effects is the appearance of one or more brown patches on the surface of the fruit. Also, a sudden increase of the respiration rate is thought to mark the transition (KIDD and WEST, 1945; JORGENSEN and KONDO, 1967). The present study showed that the two effects do not necessarily coincide; in 37 specimens of Jonathan apple, each showing both of the effects, no less than 31 specimens showed the one effect several weeks before or after the other. The average lapse of time between the two differed from season to season, which indicates either a dissimilarity of host-parasite relationships, or even the non-existence of a common cause.

As to a possible proportional effect on respiration rate of a small lesion on an apple skin: a circular lesion of 2 mm ⌀ and 1 mm depth (which would be readily observed on an apple of 65 mm ⌀) would occupy no more than 0.02% of the surface of the fruit, and no more than 0.002% of its volume; consequently, the direct effect of a change in the respiration rate of the lesion would be imperceptible, even if the respiration rate of the skin per unit weight was 18.5 times higher than that of the flesh, such as observed by HACKNEY (1945).

SOFTENING

Methods

The evaluation of mechanical properties, as described in recent literature, is of little use to our second topic, the study of trends of softening of fruits and vegetables during storage. Related interests found in recent literature may be classed under (i) testing of instruments, (ii) evaluating of some index of maturity, (iii) possibilities regarding mechanical harvesting and handling, and (iv) serving the food preservation industry.

MOHSENIN, COOPER and TUKEY (1963) state – with apparent resignation – 'In practice (...), mechanical tests of textural evaluation have normally involved the use of arbitrarily described methods, the instrumental design occupies the place of first importance, practical correlation a second place, and the fundamental significance a poor third. The correlation factors have been used without knowing fundamentally what is being measured and why the products behave as they do when subjected to mechanical forces. As a result of this, despite the numerous and specialized texture-measuring devices, the mystery of texture evaluation still remains'.

Nevertheless, the development of new experimental designs need not be discouraged, according to MORROW and MOHSENIN (1966): 'Very few studies have been conducted on agricultural products utilizing dynamic measurement methods, but such an approach appears highly desirable for future investigations'. This desirability is demonstrated in the few results discussed in section 5.3.

The technical side is exposed again by MORROW and MOHSENIN (1966): 'It is realized that an intact product such as a fruit violates all of the fundamental assumptions of homogeneity, isotropy and continuity that are normally required in solving elementary materials science problems. Disregarding the violation, however, it is possible to consider a 'black-box' approach to modulus evaluation'. The black-box approach apparently also satisfies our intention to compare trends.

BOURNE, MOYER and HAND (1966) studied reversible and reproducible compressions, at a rate of 1 cm/min, of a single apple between flat surfaces. Moreover, they discussed a few terms that ought not to be used indiscriminate: 'Relaxation occurs after each loading step, and recovery occurs after each unloading step. Whether the apple will relax or recover at any given load depends on the loading history immediately prior to the time of measurement. At a 15-kilogram load, for instance, the apple relaxes if the load has just been increased from zero, but the same apple shows recovery at 15 kg if it has just been unloaded from 30 kg. Relaxation and recovery continue to occur after many loading and unloading cycles so long as the load does not approach the yield point of the apple'. Apparently, the INSTRON machine used allowed a force to be measured while it changed without movement of the crosshead. In our investigations with the 'P-120 METTLER' compressor, the opposite occurred; relaxation and recovery of a test fruit under load caused the plunger to move virtually without changing the force.

In the dynamic measurements discussed with fig. 31, the compressions occur at a greater speed than the recoveries; thus, the alternate loading-unloading is inducing an ever increasing compression. The opposite occurs whenever compression at a constant load is followed by dynamic measurements, in the way shown in fig. 39; amplitudes at compression appear smaller than at recovery, at the times C, F, H and L. In any series of dynamic measurements the amplitudes can be made equal by means of a suitable change of the load at compression or of the load at recovery. In that way, the equal amplitudes have been obtained, which mark the onset of each of the series shown in figs. 26 and 36 to 39.

In the present work, no use has been made of mechanoelectric devices to measure softening rates (apart from the studies on resonance and on vibrational energy transmission that were discontinued as soon as they were found unprofitable), because literature did not seem to contain anything that might succesfully compete (both economically and with regard to reliability) with the instruments described here. The apparatus described by MOHSENIN (1963) can be used with strain gauges and linear differential transformers; these seem the most practical units in case one wants electric currents and voltages to do the work, instead of a mechanical unit such as the METTLER compressor, or instead of liquid flow and hydrostatic pressure, such as in the other units described here.

Results

As to softening (of tomatoes), daily determinations repeated by means of the standard bellows method, revealed that the depth of compression advanced at a rate less than 0.03 mm/day. If a constant load on a test fruit had been found to cause uninterrupted compression of 0.03 mm/day, one would not hesitate to take that as a measure of shrinkage. In our standard bellows determinations, however, shrinkage cannot interfere, as the depth of any compression is measured with regard to the point of ultimate contact that exists at the moment between the bellows' plunger and the test fruits' surface. Apparently, the rates of decline shown in fig. 33 are a non-biased measure of softening. This conclusion is supported by the high value of the rank correlation found between firmness values and colour grade numbers. Firmness values in green fruits turning red change more than those in red fruits becoming overripe (see table 1), the advance of compressions being respecitvely 1.12–0.82 = 0.30 mm, and 1.22–1.12 = 0.10 mm. This contradicts rule-of-thumb experience.

The following observations support the conclusion that tomato tissue is of a nearly perfect elasticity, combined with a very high viscosity:

a nearly rectilinear relationship was found between a very slowly increasing load and the resultant deformation,

recovery was proportional, about 40% in the first minute, irrespective of the depth of the preceding compression;

any indentation of the fruit's surface, leaving the skin intact, disappeared almost completely after some time;

in dynamic measurements, a loading-unloading cycle showed time-deformation characteristics similar to those of an elastic gel; that feature could be changed into viscous flow by killing the fruit tissue by means of an electric current.

THE YIELD POINT

The yield point is another source of information on the storage life of fruits. It stands apart from CO_2-production and softening, in that the trend it follows cannot be measured unless the test fruit is disturbed. MOHSENIN, MORROW and TUKEY (1965) consider the yield point test to be non-destructive, which is open to question. Anyhow, the yield point test may be safely presumed to provide information where other firmness tests failed.

A definite yield point in tomato was found neither with a steady increase of the load, nor with intermittent increase. Evidence regarding the non-existence of a yield point in tomato is not obtained unless the tests are carried out up to the point of rupture of the tomato's skin. In the present study, the tests were discontinued after compression had proceeded a few millimeters, without rupture of the skin. Moreover, precautions have been taken to have the tests carried out on spots that to all probability had not been bruised before.

With the aid of a METTLER balance compressor, a definite yield point was found in an apple fruit. It appeared remarkably consistent when determined on different spots of the apple surface. On the other hand, the point of rupture of the skin, which is commonly called the penetrometer value and which is of no consequence in a study of non-disturbing methods, appeared very inconsistent.

FINAL CONCLUSION

Apparently the evaluation of trends in respiration rates and softening of fruits and vegetables in refrigerated storage requires:

numerous specimens as test objects;

a close control of test conditions, which exclude uncontrollable interactions between test objects;

experimental conditions that keep the experimental error well below 1 %;

immediate recognition of spontaneous deviations from trends.

These conditions fulfilled, further investigations cannot fail to enlarge the scientific background of the refrigerated storage of fruits and vegetables.

7. SUMMARY

Physiological functions of a specimen of fruits or vegetables in storage follow trends that are either 'normal' or 'abnormal'. Normal functioning will produce changes that are known to occur under optimal storage conditions, and that result in an optimal market quality. Abnormal functioning may lead to the appearance of the typical symptoms of a storage disease. The beginning of abnormal functioning is supposed to be marked by a deviation from one or more of the normal trends.

To detect possible deviations, trends of physiological functions of stored produce were followed. A new type of conditioning machine was used, a description of which is given in chapter 3. The apparatus allows a number of test objects (single specimens) to be viewed from all sides, without disturbing their storage conditions, and without interfering with other determinations made on the test material. The storage conditions can be kept independent from the temperature, the composition and the barometric pressure of the outer atmosphere.

One of the trends that can be followed in the conditioning machine is the CO_2-production rate. A new method is described (chapter 4) that allows the CO_2-production rates of seventy apples, kept at low storage temperature, to be recorded individually, within four hours, with an experimental error not exceeding 2%. From each apple in turn, a stream of air carrying respiratory CO_2 is mixed with a flow of titrant, a solution of potassium methanolate in acetone and methanol, 0.001 to 0.004 N. The supply of titrant is adjusted until equivalent with the rate of CO_2 absorbed. Adjustment is made by means of a variable air pressure, which is read on a manometer. For a number of rates of CO_2 supply, the 'equivalent manometer value' was compared with gravimetric measurement of CO_2; the relation between the two was linear, so that the 'equivalent manometer values' could be used as a measure of CO_2 production rates of individual apple specimens.

Deviations from 'normal' trends of CO_2 production of Jonathan apples were found to start either spontaneously (i.e., apparently not induced by an environmental factor) or to be induced by a transient change in the storage temperature. In one season, spontaneous deviations were found to start several weeks before the first symptoms of decay appeared on the surface of the fruit; in another season, this sequence was reversed. This might indicate either that the two effects do not have a common cause, or that host-parasite relationships vary from one season to another (section 4.2.2.2).

A transient thermal stimulus was found to induce deviations that were either transient or apparently irreversible (section 4.2.2.3). The reversible character of a deviation would show within a few days or weeks, after which the previous trend would be resumed. Irreversible deviations sometimes brought to light differences between specimens that had followed identical courses before the stimulus was applied. Also, specific differences were found: Boskoop apples showed a deviation one day later than Jonathan apples, and less pronounced.

In an Appendix, a description is given of a new closed container method for the determination of CO_2-production rates of single specimens and of composite samples of apples and other objects.

New methods have also been tried to study *softening* of fruits in store. The 'METTLER Compressor' is a precision balance provided with a plunger (section 5.2.2). The 'Bellows Compressor' is a (plastic) bellows provided with a plunger; it operates by means of a variable hydrostatic pressure read on a liquid manometer (section 5.2.3).

Determinations with these compressors necessitate the transfer of test fruits from storage conditions to the apparatus.

In case the test fruits should be left in the same environment, the 'Hydraulic Sweller' and/or the 'Hydraulic Belt' are alternatives. The one can be fixed inside a fruit, the other around a fruit. Both operate by means of a variable hydrostatic pressure which causes a liquid meniscus in a transparent capillary tube to move; the displacement serves as a measure of the induced deformation. Deformations in the order of 0.005 to 0.04 mm/day were established, the experimental error being less than 0.001 mm. Such deformations, when found with the aid of a hydraulic belt, seem to be caused by both softening and shrinkage of the test fruits. Hydraulic swellers allowed the beginning of internal rotting in apple and pear fruits to be read from a distance.

The Bellows compressor, showed different manometer readings representing 'firmness values' on the equator of tomato fruits: over cross-walls, firmness values were 4.7% $\pm$ 1.16 higher than over locules (section 5.3.4). The tomato blossom end was found to provide firmness values that were in close correlation with the grade of colouring of the fruits (section 5.3.5). In the course of time, blossom-end firmness values of tomato fruits (as determined with the bellows apparatus) gradually decreased, parallel with the change in colour (table 1). The plunger of the compressor gained 0.007 to 0.025 mm/day, the experimental error being 0.01 to 0.02 mm. These results are not affected by shrinkage of the fruits.

Tests have been made on objects that changed their consistence, within a few minutes, while being subjected to alternating pressures. In a jellifying mixture, the alternating time-deformation curves changed from rectilinear to asymptotic whereas the opposite tendency occurred in fruits that were subjected to an injurious electric current (section 5.3.7).

With the aid of a METTLER Compressor, different spots on the surface of an apple fruit were tested. They appeared to be identical as to their yield point. but different as to the point of rupture of their skin (section 5.3.2). In tomatoes, no yield point appeared, although the test resulted in a distinct indentation in the surface of the test fruit.

8. APPENDIX

THE 'ROCKER' METHOD FOR MEASURING CARBON DIOXIDE PRODUCTION IN FRUITS

Basically, this is a closed-container method; respiratory CO_2 is absorbed in baryta solution, the residual alkalinity of which is titrated with acid.

Samples of fruits are enclosed in thermostated containers, each of which is equipped with a small aspirator pump. Between measurements, each aspirator sweeps fresh air over a sample. During absorption, an aspirator, a container and an absorber are interconnected in series by means of flexible tubing. In the closed circulatory system the carbon dioxide content of the circulating air changes until equilibrium has been reached between (A) rate of production of the sample, and (B) rate of absorption in the scrubber. Measurement of A is started not before B is constant, in other words, not before a reverse linear relationship is found between the time of exposure and the amount of acid needed for the titration of the baryta. Usually, one or two hours will suffice for equilibrium to be reached.

In preparation of a series of routine measurements the following essential factors are to be evaluated: (i) the time needed to reach equilibrium; (ii) the time after which oxygen depletion in the closed system necessitates renewed ventilation; (iii) optimal conditions of absorption, e.g. appropriate volume and titer of the baryta to be used in the absorber.

The absorber is a 200 ml Erlenmeyer flask, closed with a two-hole rubber stopper. The two holes are provided with short pieces of glass tubing, which do not extend below the stopper. Above the stopper the tubes extend a few centimeters; they are interconnected with a short piece of rubber tubing that is securely fastened to one of them and loosely fitting the other. The latter is disconnected while the absorber is incorporated into the circulatory system mentioned before.

Of this type of absorber some dozens are made available, having all the same model and volume. If different sizes and/or shapes are used alternately, results are much less consistent.

By means of a high precision automatic pipette (fig. 40) an appropriate volume of baryta (e.g. 20 ml, about 0.1 N) is transferred to a flask. During that operation CO_2-free air (about 1 liter/min) is blown into the flask to exclude contamination with atmospheric carbon dioxide. After filling, the flask is closed and set aside until needed.

While functioning in a circulatory system, an absorber is shaken mechanically with a frequency of about 80 to 100 cycles per minute and with an amplitude of 4 to 5 cm. This prevents splashing as well as formation of a crystalline layer of carbonate on the baryta. Several flasks are shaken simultaneously while

[1] Valuable assistance in the development of this method was obtained from Mr. Q. P. VAN DER MEER.

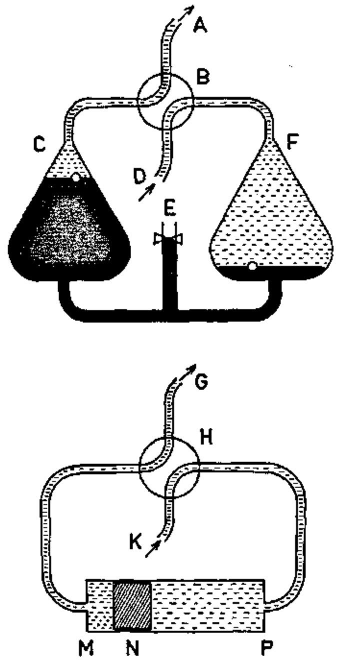


FIG. 40. Automatic dispensing pipettes.
All-glass type G-P is commercially available (Liquomaat, capacities not adjustable, ranging from 0.5 ml to 50 ml). A hydrostatic pressure difference should be upheld between the liquid inlet K and the outlet G; it forces glass piston N to either end M or P of the cylinder as soon as stopcock H is turned. At each turn a given volume of liquid is dispensed from G. The system is fed from a supply bottle that is put on a support to create a hydrostatic pressure difference. The greater the pressure difference, the more rapid the system works but the more liquid will leak past the piston after the latter has been stopped at M or at P.

The "Twin" pipette A-F originated before the Liquomaat had been found commercially available. Here the piston is an adjustable volume of mercury which is moved by force of a hydrostatic pressure difference to be upheld between D and A. Movement starts as soon as stopcock B is turned, and is stopped by a ball (steel or glass) that cannot pass through C or F and that is floating on the mercury surface. The two balls are to be introduced into the system via stopcock E which also serves for the adjustment of the volume of mercury. This volume is inversely related to the volume of liquid to be dispensed from D to A. The system can withstand a hydrostatic pressure difference of more than one meter which allows very rapid dispensing. At high dispensing rates, a little liquid can be caught between the rising mercury and the inner surface of the pipette; the conical shape of the pipettes exposes the least possible surface and keeps the standard deviation down to a few cubic millimeters. Leakage may occur i.e. the mercury is not stopped definitely at C or at F, if (i) the floating ball is not exactly spheric, (ii) the inside surface of the pipette top has not been ground conical, or (iii) the mercury surface (D and H) is not clean.

clamped to a horizontal bar (one or two meters long) that rests on roller bearings and is kept in longitudinal oscillation with the aid of a 50 watt motor provided with a gear box and an eccentric movement.

As soon as the circulatory systems have been closed, absorption is started by simultaneously starting the aspirators and the rocking movement. As will be understood, absorption of CO_2 is accomplished not by bubbling through the solution but by blowing the air over it. So any losses due to splashing and foaming are prevented, which adds considerably to the reproducibility of the results.

When the predetermined exposure time has passed, both the air circulation and the rocking are stopped and the absorbers are disconnected and closed. At that moment an unabsorbed rest of CO_2, which may amount to 0.5 mg, is present in the air volume of the flask; this rest should be allowed also to be absorbed in the baryta. If the flask is kept standing this will take half an hour; if the flask is kept rocking it may be accomplished within a few minutes.

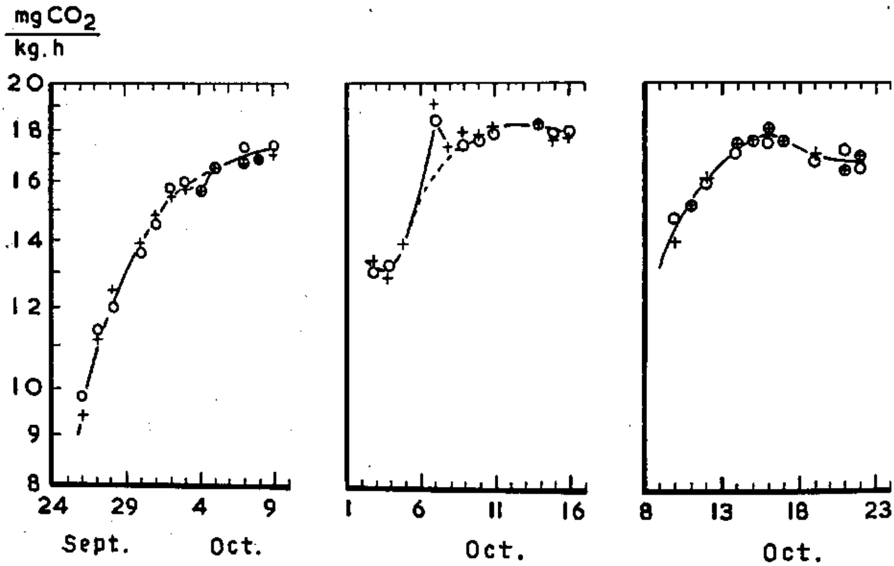
Immediately after the flask has been disconnected, either a fresh absorber is connected into the circulatory system or the sample is ventilated with fresh air. An absorption period is reckoned to begin at the moment the previous circulation or ventilation was stopped.

The baryta is titrated with 0.1 N potassium biphtalate solution, with thymolphtalein (0.5% in ethanol) as an indicator. During titration, as during filling, contamination with atmospheric CO_2 is prevented with the aid of a stream of CO_2-free air. The titration of an absorber flask takes no more than 2 minutes, as there is not a residue of splashed-up baryta to be rinsed down and, moreover, the entire content of an absorber is titrated, so no time has to be spent with taking and transferring aliquot volumes of baryta.

Variance of results may be due to a number of factors:

- fluctuations of exposure time. Optimal exposure time is between 1 and 2 hours; fluctuations of half a minute are then unsignificant.
- unbalance between CO_2-production and absorption. This may be due to the measuring having been started before a proper equilibrium had been reached, or because an absorber has been exposed until the baryta was more than 60% saturated with CO_2, which causes the absorption rate to decline.
- titration error. A piston type burette with digital reading is recommended for the purpose.
- CO_2-loss from the circulatory system (leakage, diffusion) or contamination from outside air (inward diffusion may occur when the CO_2-level of the outside air is high, as may occur in fruit storage cabinets).
- actual fluctuations of the CO_2-production of the sample, owing to either physiological causes or to improper ventilation; the latter is avoided by properly directing the flow through the sample, by a high air flow rate (which in a closed circulatory system will *not* impede absorption) and by loosely packing the sample (no air pockets).

With proper attention to these factors the mean coefficient of variation of results was mostly less than 2%, with composite samples as well as with single specimens (fig. 41 and table 2; determinations made by Q. P. VAN DER MEER).



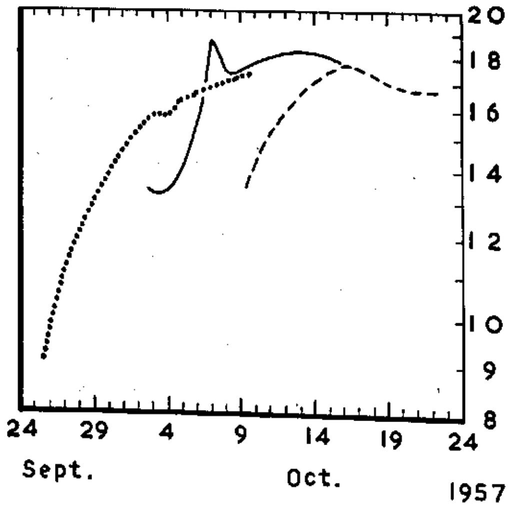


FIG. 41. CO_2 production of Jonathan apples as related to date of harvest (September 24 October 1 and 8, 1957) and as determined with the "Rocker" method, using the "Twin" dispensing pipette. Duplicate samples (indicated with o and with +) of 12 specimens each stored at 12°C. The three trend lines have been combined in the lower diagram. The peak occuring at October 7 is apparently due to some technical failure.

TABLE 2. CO_2-production of six individual apple fruits (Cox' Orange Pippin) during three consecutive periods of two hours each; before measuring, absorption was equilibrated in 1½ hour; temperature about 4°C.

Apple no.	mg CO_2 absorbed during period no. I	II	III
1	1.94	1.94	1.96
2	1.91	1.87	1.87
3	1.91	1.87	1.91
4	2.44	2.44	2.53
5	2.16	2.18	2.11
6	1.98	2.00	1.98

9. ACKNOWLEDGEMENTS

The help and encouragement I got, from the start of this work, in 1949, up to the present publication, is acknowledged to its full extent, but only part of it can be mentioned here.

The late Ir. A. K. ZWEEDE, and the present superintendent of the Sprenger Institute, Ir. T. VAN HIELE, made it possible that the investigations were carried out.

Prof. Dr. E. C. WASSINK accepted the present publication as a doctor's thesis, and gave the impulse to the development of new techniques for measuring softening.

Dr. R. G. TOMKINS, of Ditton Laboratory, Maidstone, England, gave expert advice on a previous draft of the manuscript, and was kind to this Dutchman's English.

JAKOB APELAND, Vollebekk, Norway, as a guest research worker in the Sprenger Institute, spent many months to aid my investigations on resonance in fruits.

My need of various instruments and apparatus of unusual design was satisfied by the staff of the Wageningen Service Institute for Applied Mechanics and Technical Physics in Agriculture, especially Ir. G. BOREL. Also, Mr. H. J. DE ROOY performed expert glassblowing.

Skilful and perseverant assistance in the development of new techniques and in the compilation of multitudinous research data was given by Mr. T. HONKOOP. Also, Miss H. W. STORK and Ir. Q. P. VAN DER MEER, F. COURBOIS and J. LANGERAK must be mentioned in this connection.

10. SAMENVATTING

BEPALING VAN HET VERLOOP VAN DE ADEMHALINGSINTENSITEIT EN HET ZACHT WORDEN VAN VRUCHTEN VAN APPEL EN TOMAAT NA DE OOGST

Verondersteld wordt, (i) dat de fysiologische functies van vruchten en groenten na de oogst hetzij een 'normaal' of een 'abnormaal' verloop hebben, (ii) dat een normaal verloop leidt tot optimale bewaarresultaten, (iii) dat een abnormaal verloop kan leiden tot de symptomen van een bewaarziekte, (iv) dat een afwijking van het normale verloop van één of meer functies kan worden beschouwd als het eerste stadium van een bewaarziekte, (v) dat zulk een afwijking, indien tijdig gesignaleerd, kan dienen als een waarschuwing – in verband met verdere opslag van het product – ofwel als een aanleiding om andere aspecten van een beginnende bewaarziekte te gaan onderzoeken, (vi) dat men te meer kans heeft tijdig te signaleren naarmate meer objekten gelijktijdig in onderzoek zijn, naarmate de – experimentele – opslag van de proefobjecten beter aan dat doel is aangepast en naarmate tevens de proefobjecten meer doelmatig worden onderzocht.

Laboratorium-apparatuur voor geconditioneerde bewaring wordt beschreven (hoofdstuk 3). Zij biedt plaats aan 72 appels, die onafhankelijk van elkaar kunnen worden bestudeerd. De temperatuur, de samenstelling en de barometerdruk van de atmosfeer rond de proefobjecten kunnen voor onbepaalde tijd naar willekeur worden ingesteld; tevens wordt het milieu niet verstoord wanneer de proefobjecten aan een onderzoek worden onderworpen, althans wanneer het onderzoek daartoe geëigend is.

Onder andere kan de CO_2-productie van proefobjecten in het bewaar-apparaat worden gemeten. Daartoe wordt een nieuwe methode beschreven (hoofdstuk 4), waarmee zeventig bepalingen binnen vier uur kunnen worden afgewerkt met een onnauwkeurigheid minder dan 2%. De methode berust op rechtstreekse titratie van het CO_2 dat door een proefobject (appel) wordt geproduceerd. Een stroom afgewerkte ademhalingslucht van een proefobject wordt intensief gemengd met een stroom titreervloeistof, die zodanig wordt geregeld dat zij equivalent is aan het geabsorbeerde CO_2. De regeling geschiedt met behulp van een variabele luchtdruk, die tevens dient als maat voor de aanvoer van CO_2.

Afwijkingen van het normale verloop van de CO_2-productie van Jonathan appels bleken hetzij spontaan op te treden (d.w.z. zonder enig aanwijsbaar verband met bijvoorbeeld een verstoring van het milieu), ofwel onmiddellijk te volgen op een voorbijgaande wijziging van de bewaar-temperatuur (zie 4.2). Spontane afwijkingen vertoonden zich in 1958–59 gemiddeld 31 dagen ná de eerste waargenomen symptomen van stek op het oppervlak van de vruchten; in 1960–61 gemiddeld 2 dagen er vóór, en in 1961–62 gemiddeld 20 dagen tevoren. Er is dus hetzij geen causaal verband tussen het optreden van stek en van een plotselinge toename van de CO_2 productie, ofwel zulk een verband wisselt met de betrekkingen tussen gastheer en pathogeen (zie fig. 18).

Een voorbijgaande verstoring van het milieu, een tijdelijke wijziging van de bewaar-temperatuur, had tot gevolg dat de CO_2 productie van een appel afwijkend werd, hetzij tijdelijk ofwel kennelijk irreversibel (zie fign. 19 en 20). Specimina die vóór de verstoring eenzelfde verloop van de CO_2 productie te zien gegeven hadden, gingen daarná op uiteenlopende wijze verder. Hieruit zou men kunnen afleiden dat een voorbijgaande verstoring van het milieu een onderscheiding mogelijk maakt tussen kennelijk niet vergelijkbare proefobjecten.

De verandering van consistentie, het zachter worden (van vruchten en groenten na de oogst) was het tweede aspect van het onderzoek, waarvoor eveneens nieuwe methoden werden beproefd. De 'METTLER Compressor' is een zogenaamde Bovenweger, waaraan een stempel is bevestigd (zie fig. 21). De 'Balg Compressor' (zie fig. 22) bestaat uit een plastic balgje, waaraan eveneens een stempel is bevestigd die door middel van een variabele vloeistofkolom op en neer bewogen wordt. Met beide apparaten kunnen goed reproduceerbare metingen worden uitgevoerd, waarbij echter het proefobject tijdelijk uit zijn bewaar-milieu moet worden genomen. Dit nadeel bestaat niet bij twee andere hydraulische meetapparaten, de 'Hydraulische Gordel' en de 'Hydraulische Zweller'. Deze kunnen voor onbepaalde tijd mechanisch gekoppeld worden aan een vrucht, en op afstand worden bediend en afgelezen. Zij brengen in het proefobject een deformatie teweeg met behulp van een regelbare vloeistofdruk, die zo nodig wordt aangevuld met een regelbare luchtdruk. De verplaatsing van een meniscus dient als maat voor de vervorming. Met geen van beide instrumenten kan het verschil tussen zachter worden en schrompelen van het proefobject worden afgelezen. De combinatie van die twee effecten leidde er toe dat de diameter van een tomaat in een hydraulische gordel schijnbaar afnam met 0,01 tot 0,08 mm/dag, een proces dat met een onnauwkeurigheid van minder dan 0,001 mm kon worden vervolgd. Door middel van hydraulische zwellers kon het begin van inwendige rotting in appels en peren op afstand waargenomen worden.

Met de balg compressor werden reeksen hardheidsmetingen uitgevoerd op tomaten. De equator van een tomaat vertoonde uiteenlopende hardheden; op het kruispunt met een tussenschot was de vrucht 4,7% $\pm$1,16 harder dan op het kruispunt met de mediaan van een hok. Het kroonlitteken (de neus) bleek een geschikte plaats om 'de hardheid' van een vrucht te meten; de gevonden waarden bleken hoog gecorreleerd aan 'hardheden' van andere plaatsen van de vrucht en konden dus gelden als representatief; tevens was de afname van de 'hardheid' van het kroonlitteken hoog gecorreleerd aan de verandering van de kleur van de vrucht, die met een puntenstelsel werd gewaardeerd. Het geleidelijk zachter worden bleek uit een aantal reeksen hardheidsmetingen met de balg compressor waarvan de stempel met elke volgende metingen dieper indrong. De toename bedroeg 0,007 tot 0,025 mm/dag, met een onnauwkeurigheid minder dan 0,004 mm. Metingen met de balg compressor en met de METTLER Compressor worden niet beinvloed door het krimpen van het proefobject.

Voornoemde meetresultaten betreffen het verband tussen een kracht en de daardoor teweeg gebrachte mate van vervorming (indrukking). Van gelijk belang is de snelheid waarmee een vervorming tot stand komt, een kenmerk waarmee rheologische verschillen kunnen worden onderscheiden. Desbetreffende metingen werden verricht door middel van rhytmische vervormingen, teweeg gebracht met een hydraulische zweller of -gordel, mechanisch gekoppeld aan een proefobject, en afgelezen als bewegingen van een vloeistof-niveau. Die bewegingen waren eenparig wanneer het proefobject een viskeuse vloeistof was, en werden asymptotisch terwijl die vloeistof geleerde. Een wijziging in omgekeerde zin voltrok zich toen als voorwerp van onderzoek een tomaat fungeerde, die plaatselijk beschadigd werd door een electrische stroom.

Met behulp van de METTLER compressor en de balg compressor werd in appel en tomaat nagegaan welke indrukking leidt tot kneuzen (Yield point). Drie verschillende plaatsen op het oppervlak van een appel vertoonden een zelfde yield point; daarentegen waren sterk uiteenlopende indrukkingen nodig om de schil te doorbreken. Tomaten vertoonden geen duidelijk yield point, alhoewel de proefobjecten zodanig ingedrukt werden dat de afdruk van de stempel nog vele uren zichtbaar bleef.

In een Appendix wordt een methode beschreven ter bepaling van de CO_2 productie van appels in een gesloten systeem.

11. LITERATURE CITED

ALEXANDER, B. and F. J. FRANCIS; A method for gas chromatographic respiration studies and results with prepeeled turnips. Proc. Amer. Soc. Hort. Sci., **84** (1964), 519–526.

ANON. Technisch Verslag 1961–1962. Comité voor Onderzoek op de Bewaring van Fruit en Groenten, Sectie I, Louvain, Belgium. (1962), 71 pp.

BAIN, J. M. and F. V. MERCER; Organization resistance and the respiration climacteric. Austral. J. Biol. Sci. **17** (1964), 78–85.

BEIER, W. and E. DOERNER. Die Physik und ihre Anwendung in Biologie und Medizin (I). Thieme, Leipzig, 1956, p. 221.

BIALE, J. B.; Postharvest physiology and biochemistry of fruits. Ann. Rev. Plant Physiol. **1** (1950), 183–206.

BLOM, L. and L. EDELHAUSEN; Direct titration of carbon dioxide. Analytica Chimica Acta **13**, (1955), 120–8.

BOEKE, J. E.; Preliminary studies on the anamnesis of storage diseases in fruits and vegetables. Annexe 1961–1. Bull. Instit. Internat. du Froid (1961), 17–22.

BOURNE, M. C.; Studies on punch testing of apples . Food Technology **19** (1965), 113–115.

BOURNE, M. C., J. C. MOYER and D. B. HAND; Measurement of food texture by a universal testing machine. Food Technology **20** (1966), 170–174.

CLAYPOOL, L. L. and R. M. KEEFER; A colorimetric method for CO_2 determination in respiration studies. Proc. Amer. Soc. Hort. Sci., **40** (1942), 177–86.

CLAYPOOL, L. L., E. C. MAXLE and PAUL ESAU; Effect of aeration rate on the respiratory activity of some deciduous fruits. Proc. Am. Soc. Hort. Sci., **66** (1955), 125–134.

CURTIS, H. J.; Bioelectric measurements, p. 233–70. *In* UBER, F. M.; Biophysical research methods. Interscience Publ. New York & London. (1950).

DE PLATER, C. V. and C. G. GREENHAM; A wide-range A. C. Bridge for determining injury and death. Plant Physiol. **34** (1959), 661–667.

FALK, S., C. H. HERTZ and H. I. VIRGIN; On the relation between turgor pressure and tissue rigidity. I (Experiments on resonance frequency and tissue rigidity). Physiol. Plant., **11** (1958), 802–817.

GAC, A.; Contribution à l'étude de l'influence de l'humidité relative et de la vitesse de circulation de l'air sur le comportement des fruits cueillis. Thèse Ingénieur Docteur, Sorbonne, Paris, (1955). 201 pp.

GOODERHAM, W. J. ;Soap film calibrators. J. Soc. Chem. Ind. Transact. **63** (1944), 351–2.

HACKNEY, F. M. V.; Studies in the metabolism of apples. VI: Preliminary investigations on the respiration of sliced apple tissue. Proc. Linnean Soc. N. S. Wales **70** (1945), 333–345.

HAMSON, A. R.; Measuring firmness of tomatoes in a breeding program. Proc. Amer. Soc. Hort. Sci., **60** (1952), 425–433.

HOBSON, G. E. (1959); A note on the measurement of the firmness of tomato fruit. A. R. Glasshouse Crops Research Institute (1958/1959) 66–68.

HULME, A. C.; Apparatus for the measurement of gaseous conditions inside an apple fruit. Journ. of Exper. Botany, **2** (1952), 65–85.

JORGENSEN, E. and E. S. KONDO; Measurement of gas exchange in woody plants. Nature **213** (1967) 413–414.

KATTAN, A. A. ;Changes in color and firmness during ripening of detached tomatoes, and the use of a new instrument for measuring firmness. Proc. Amer. Soc. Hort. Sci., **70** (1957), 379–384.

KIDD, F. and C. WEST; Respiratory activity and duration of life of apples gathered at different stages of development and subsequently maintained at a constant temperature. Plant Physiol., **20** (1945), 467–504.

KRUMBHOLZ, G. and N. WOLODKEWITSCH; Festigkeitsmessungen an Früchten und ihre Anwendungsmöglichkeiten. I. Die bestimmung der Fruchtfleischfestigkeit. Die Gartenbauwissenschaft, **17** (1943), 543–590.

LABRIQUE, J.–P.; Mesures électriques sur des cellules végétales. Bull. Acad. Roy. Belg. (Cl. Sci.), **46** (1960), 791–803.

LABRIQUE, J.–P.; Réponse électrophysiologique de plantes soumises à de brusques modifications d'éclairement (effet *on-off*). Compt. rend. séanc. Soc. Biol., **45**, (1961a), 1395–7.

LABRIQUE, J.–P.; L'électrophysiologie végétale. Rev. Quest, Sci. (1961b), 340–366.

LANDFALD, R.; Interim warming of apples stored at 0°C. Annexe 1961–1. Bull. Instit. Internat. du Froid, (1961), 105–110.

LUYET, B. J.; Variation of the electric resistance of plant tissues for alternating currents of different frequencies during death. Journ. Gen. Physiol., **15**, (1932), 283–7.

MCCOLLOGH. Lacy P.; Bruising injury of tomatoes. U.S.D.A. Marketing Research Report 513. (1962), 31 pp.

MCGONNAGLE, W. J.; Nondestructive testing. MCGRAW-HILL Book Cy., New York, (1961) xi + 455 pp.

MEER, Q. P. VAN DER, and E. C. WASSINK; Preliminary note on photosynthesis in green plum fruits. Meded. Landb. Hogesch. Wageningen, **62** (12) (1962), 1–9.

MIESSNER, H.; Fortschritte der Verfahrenstechnik. Bd. **5**, Verlag Chemie, Weinheim, (1962). p. 267.

MOHSENIN, N. N., H. E. COOPER and L. D. TUKEY; Engineering approach to evaluating textural factors in fruits and vegetables. Trans. A.S.A.E. **6** (2) (1963), 85–88+92.

MOHSENIN, N., H. GOEHLICH and L. D. TUKEY; Mechanical behaviour of apple fruits as related to bruising. Proc. Amer. Soc. Hort. Sci., **81** (1962), p. 67–77.

MOHSENIN, N. N. and H. GOEHLICH; Techniques for determination of mechanical properties of fruits and vegetables as related to design and development of harvesting and processing machinery. J. Agric. Engr. Res. **7** (4) (1962), 300–315.

MOHSENIN, N. N.; A testing machine for determination of mechanical and rheological properties of agricultural products. Pennsylvania Agric. Exp. Sta. Bull. **701**, (1963), 26 pp.

MOHSENIN, N., C. T. MORROW and L. D. TUKEY; The 'yield point' non-destructive technique for evaluating firmness of Golden Delicious apples. Proc. Amer. Soc. Hort. Sci. **86**(1965), 70–80.

MORROW, C. T. and N. N. MOHSENIN; Consideration of selected agricultural products as viscoelastic materials. J. Food Sci, **31** (1966), 686–698.

NICHOLAS, R. C.; Some observations on the use of fruit pressure testers. Michig. State Univ. Quart. Bull. **43** (2) (1960), 312–326.

NYBOM, N.; A new principle for measuring firmness of fruits. Hort. Res. **2** (1962), 1–8.

OORSCHOT, L. J. P. van and M. BELKSMA; An assembly for the continuous recording of CO_2 exchange and transpiration of whole plants. Weed Res. **1** (1961), 245–257.

PERRY, R. H., C. H. CHILTON and S. D. KIRKPATRICK; Chemical engineer's handbook. 4th ed. McGraw Hill, Nw. York, 1963.

SCHIPPERS, P. A.; Preliminary investigations into respiration and transpiration in vegetables during storage. Report **8**, Nw. Zealand Dept. Sci. Industr. Res., Otara (1965) 17 pp.

SCHWAN, H. P.; Electrical properties of tissue and cell suspensions. Advances in Biol. and Medic. Phys. **5** (1957); 147–209.

SHEPHARD, R. J.; A comparison of paramagnetic and chemical methods for the determination of oxygen. Z. angew. Physiol. **22**, (1966), 279–284.

SMITH, W. H.; A new method for the determination of the composition of the internal atmosphere of fleshy plant organs. Ann. of Botany, **11** (1947), 363–368.

TROUT, S. A. e.a.; Studies in the metabolism of apples. 1. Preliminary investigations on internal gas composition and its relation to changes in stored Granny Smith apples. Austral. J. Exp. Biol. Medic. Sci., **20** (1942), 219–30.

ULRICH, R.; Les repères du degré de maturité des fruits. Fruits d'Outre-Mer, **1** (1946), 456–61. 2nd part in Ibid, **2** (1947), 5–11.

ULRICH, R. ;La vie des fruits. Masson, Paris (1952), 370 pp.

VIRGIN, H. I.; A new method for the determination of the turgor of plant tissues. Physiol. Plant., **8** (1955), 954–962.

WENDLANDT, W. W., and J. M. BRYANT; Qualitative test for carbon dioxide. Chemist Analyst, **44** (2), (1955), 52.

WILKINSON, B. G.; Some effects of storage under different conditions of humidity on the physical properties of apples. J. hort. Sci. **40** (1965), 58–65.
WILLIAMS, M. W. and M. E. PATTERSON; Internal atmospheres in Bartlett pears stored in controlled atmospheres. Proc. Amer. Soc. Hort. Sci., **81** (1962), 129–136.
WOLF, J.; Einfluss einer vorangegangen Lichtperiode auf die Grösse der CO_2-Ausscheidung grüner (unreifer) Aepfel im Dunkeln. Planta. **50** (1958) 576–8.
YOUNG, R. E. and J. B. BIALE; Carbon dioxide effects on fruit respiration, I: Measurement of oxygen uptake in continuous gas flow. Plant Physiol. 37 (1962), 409–415.