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pectic substances in fresh and preserved fruits and vegetables

Institute for Research on Storage and Processing of Horticultural Produce
Wageningen, The Netherlands

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by

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Preface

Pectic substances are involved in several aspects of fruit and vegetable technology, e.g. texture and firmness of fresh and preserved products, clarification or cloud stabilization of fruit juices, jellying phenomena in jams and jellies, and manufacture of commercial pectins from fruit wastes.

For this reason it is not surprising that a research institute like the I.B.V.T., working on the technology of horticultural products, devoted a part of its research to the study of these interesting compounds and their behaviour in various products.

In this booklet an attempt is made to describe our present knowledge of these compounds in relation to this special field. The contents of the introductory chapters are therefore limited to what is needed for a discussion of the subject.

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J. J. DOESBURG

Stellenbosch, april 1965.

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Chapter I

Nomenclature and structure of pectic substances

In his book 'The pectic substances' (259) KERTESZ stated that fruit jelly making was practised long before pectin was discovered. The first information on water-soluble substances with a strong jellying power, occurring in fruits, was presented by VAUQUELIN (494) in 1790. The next well-known scientific publication on these substances was written by BRACONNOT in 1825 (60). In this publication BRACONNOT related the name of these substances to their jellying properties, when he derived it from the Greek word 'πηκτος', meaning to congeal or solidify (259).

Nomenclature

In the past various names have been used for the designation of the group of pectic substances and their derivatives. KERTESZ (259) has given a survey of this old nomenclature.

The increase of the knowledge of pectic substances has enabled a revision and unification of nomenclature. A first attempt was undertaken in 1926 (63). After some decades, a revision showed to be necessary as a result of the further developed knowledge of the pectic substances and therefore a new nomenclature has been accepted by the American Chemical Society in 1949 (262). This proposal has been used by many workers in the field of pectic substances, but a complete unification has not been attained. Definitions which are not completely identical with the American ones have been published by HENGLEIN (200) in 1947.

For these reasons it is necessary to point out which definitions will be used in this booklet.

Pectic substances is a group designation for those complex, colloïdal carbohydrate substances which occur in, or are prepared from, plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination. The carboxyl groups of these polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralized by one or more bases.

The term *pectic acid* is applied to pectic substances mostly composed of colloïdal polygalacturonic acids and nearly free from methyl ester groups. According to HOTTENROTH (225) the upper limit of methoxyl content is 0.8 %. *Pectates* are either normal or acid salts of pectic acid.

The term *pectinic acids* is used for colloïdal polygalacturonic acids containing more than a negligible portion of methyl ester groups. *Pectinates* are either normal or acid salts of pectinic acids.

The term *protopectin* is applied to the water insoluble pectic substances in plants from which pectic substances can be produced.

The definitions mentioned above are not completely identical with the definitions given by the American Chemical Society (262) or by HENGLEIN (200).

The description of the pectic substances corresponds with the American definition, whereas the definition of HENGLEIN includes in addition mixtures of polygalacturonic acids with accompanying substances, e.g. the frequently present arabans and galactans. In the HENGLEIN definition of 'Pektinstoffe' it is not mentioned that pectic substances have to be of colloïdal nature.

According to the American definition, pectic substances may be produced from protopectin by restricted hydrolysis, whereas in the HENGLEIN definition further claims as to protopectin structure are made. Actually the structure of protopectin is unknown and for this reason no remarks on the manners of extraction have been made in our definition.

The American description of pectic acid indicates that it has to be essentially free from methyl ester groups. Further, it is mentioned that pectinic acids under suitable conditions are capable of forming gels with sugar and acid or, if suitably low in methoxyl content, with certain metallic ions. However, not all colloïdal pectinic acids show a jellying power and therefore this remark on jellying properties has been omitted in the definition given above.

The formulae of d-galacturonic acid and of α-d-galacturonic acid are shown in Fig. 1.

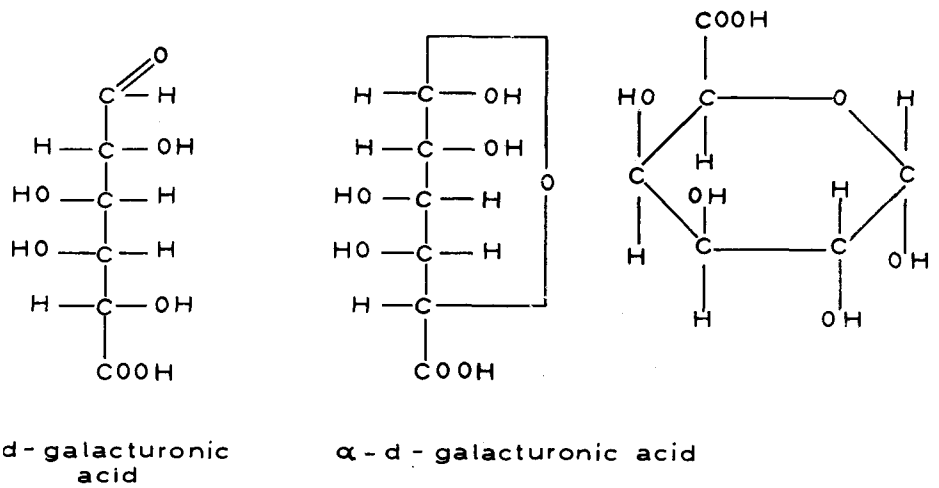


FIG. 1. d-galacturonic acid and formulae of α-d-galacturonic acid.

It has been pointed out by MCCREADY and GEE (329) that an inspection of the definitions of pectic substances and other carbohydrate polymers reveals that none is adequate for the known carbohydrates in plants. These authors have stressed that there are many known exceptions to suggested classifications and that it is likely that families of plant carbohydrates containing less than 10% and up to 100% galacturonic acid and many kinds of non-uronide sugars may occur in plants. According to ASPINALL nearly (13) all recent attempts to prepare pure galacturonans have yielded polysaccharides that contain appreciable proportions of neutral sugar constituents. Nevertheless with our present knowledge it does not seem possible to give much better definitions for pectic substances. Since the pectic substances are polymer carbohydrates containing uronic acids, the term *polyuronides* includes the pectic substances (259).

Certainly it has to be kept in mind that the limits of these definitions are rather vague, for instance an indication about the minimum polygalacturonide content is lacking and no sharp indication can be given on the minimum degree of polymerization of the polygalacturonic acids.

The definitions mentioned above are based more or less on composition and structure of pectic substances. In addition a definition is given which is founded on composition and use of some pectic substances as jellying agents.

The term *pectin* designates those water-soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels under suitable conditions.

The jellying power of pectinic acids is strongly connected with a relatively high degree of polymerization and the absence of groups attached to the pectinic acid chains which destroy the jellying power. Pectinic acids with a high degree of polymerization, which have no jellying power as the result of a high content of acetyl groups, cannot be designated as pectins. Further, the conditions needed for jellification are strongly governed by the methoxyl content. Pectins with a rather high methoxyl content show their jellying power only in the presence of a relatively high sugar and acid content, whereas gel formation by pectins with a lower methoxyl content is also possible without sugar in the presence of certain metallic ions. For this reason it is useful to distinguish two groups of jellying pectic substances, *the high-methoxyl pectins* and *the low-methoxyl pectins*. The term 'high-methoxyl pectins' is often abbreviated to 'pectins'.

A schematic survey of the interrelationship of pectic substances is presented in Fig. 2.

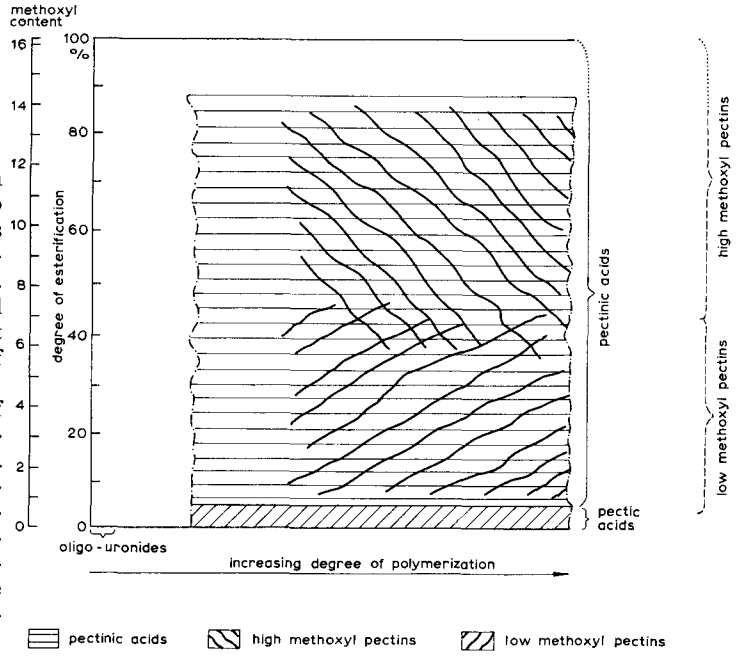


FIG. 2.
Schematic illustration of the interrelationship of pectic substances (modification of illustration by Kertesz, 259). The limit of colloidal behaviour is passed at an unknown degree of polymerization. The jellying properties of high- and low-methoxyl pectins occur at a higher degree of polymerization. Polygalacturonic acids containing 2–4 anhydrogalacturonic acid units are mentioned oligo-uronides.

Structure

According to the definitions given above the main components of the pectic substances are colloïdal polygalacturonic acids, in most cases with a part of the carboxyl groups esterified with methanol. The structure of a part of a chain of α-1,4-linked D-galacturonic acid units is shown in Fig. 3.

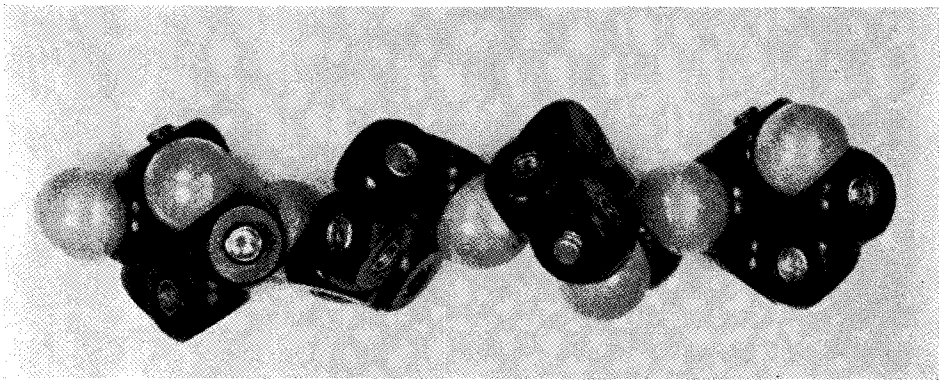


FIG. 3. Model of polygalacturonic acid, showing three fold screw symmetry (Deuel and Stutz, 119).

The formula of a part of a polygalacturonic acid molecule, partly esterified with methanol, is presented in Fig. 4.

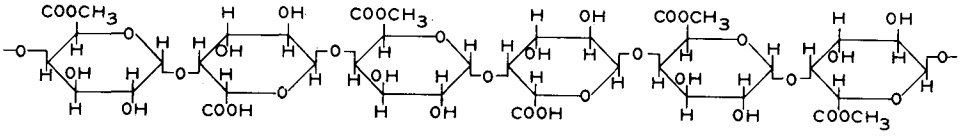


FIG. 4. Part of polygalacturonic acid molecule, partly esterified with methanol.

The measure of esterification of pure galacturonic acids may be indicated by the methoxyl (CH_3O) content or by the *degree of esterification* which represents the number of esterified carboxyl groups calculated as the percentage of the total number galacturonic acid units. When the carboxyl groups in pure polygalacturonic acids are all esterified the methoxyl content is 16.32% and the degree of esterification 100%. In Table 1, more data are given on the relation between methoxyl percent and degree of esterification of partly esterified pure polygalacturonic acids.

The upper limit of methoxyl content of pectinic acids, extracted from natural sources, was seldom higher than about 13.5%. In experiments by VOLLMERT (498), it has been shown that fully methylated pectinic acids can be prepared by treatment with diazomethane.

The *equivalent weight* is also dependent on the degree of esterification (Table 1) and can be defined as the number of grams of pure polygalacturonic acids which corresponds with an equivalent of free carboxyl groups. When the degree of esterification is x% the equivalent weight can be calculated from the formula:

$$\text{eq. weight} = \frac{176.(100—x) + 190.x}{(100—x)} = \frac{17600 + 14.x}{(100—x)}$$

TABLE 1. *Relation between degree of esterification, methoxyl content and equivalent weight of pure pectinic acids.*

Degree of esterification	Methoxyl content	Equivalent weight
0 %	0,00 %	176
10	1,63	197
20	3,26	224
30	4,90	257
40	6,53	303
50	8,16	366
60	9,79	461
70	11,42	619
80	13,06	936
90	14,69	1886
100	16,32	∞

The reducing power of pectic substances is caused by the reducing aldehyde groups on the terminal units at one end of the polygalacturonic chains. The reducing groups of the other monomer units are engaged in linking the component monomers. However, the information on the occurrence of *reducing end groups* is rather confusing, which may be caused by unknown structural irregularities as well as by methodological difficulties.

The molecular weight of pectic substances from various sources, but mainly from flax, sugar beets, apples and citrus fruits, has been the subject of numerous investigations. The results are influenced by the manner of extraction and method of measurement of molecular weight (determination of amount of reducing end groups, osmotic pressure, viscosity, sedimentation velocity). The molecular weights, which have been recorded, range from about 10,000 to about 400,000.

KERTESZ (259) states that brief consideration is sufficient to show the disharmony between the results of different authors. The values obtained may be weight or number averages and even within either of these groups may vary according to the method of determination. Further, as shown by SCHNEIDER and FRITCHI (420), in every sample, molecules with different molecular weight can be present and the averages found will depend on the sizes of the component members and their proportional distribution. Similar results were obtained by HERI (205a) who reported the presence of three fractions with a different molecular weight in a sample of pure citrus pectin.

According to our definitions the greater part of the pectic substances consists of anhydrogalacturonic acid units in a chain-like combination and partly esterified by methanol and partly or completely neutralized by one or more bases.

Up till now it has not been possible to prepare protopectin in an unchanged form from plants (245). For this reason the present knowledge of the pectic substances

has been derived from observations on pectic substances obtained by extraction with various reagents under different conditions. The reagents used and the conditions of extraction are based largely on empirical procedures since the chemical and physical factors that are responsible for the limited solubility of native pectic substances are not known completely.

The crude alcohol precipitates of pectic substances extracted from plants may contain 20–50% by weight of non-uronide matter, determined as ash, nitrogenous constituents, polysaccharides (mostly glucosans and hemicelluloses) and other substances. However, it is well established that such precipitations of pectic substances can be purified to a rather great extent by manifold repeated precipitation from aqueous solutions by ethanol (32, 216, 217, 375, 421). This is an indication that only a part of these constituents in the first precipitate may be attached to the polygalacturonic chains by primary covalent bonds, the remainder is held by secondary forces or merely as a physical admixture.

The amount of covalently linked non-uronides seems to vary considerably. BISHOP (48) reported that pectic substances from sunflower heads extracted under very mild conditions appeared to be pure polygalacturonides. MCCREADY and GEE (329) when analyzing purified pectinic acids from several fruits, carrots, sugar-beets and pea pods found, after partial acid hydrolysis, various sugar contents ranging from 8-25%. The amount of non-uronide matter in pectic substances is influenced by the manner of extraction as well as by the manner of precipitation.

BOCK (54) has pointed out that purification of pectic substances by alcohol (or acetone) precipitation is due mainly to a fractionating effect on organic ballast materials rather than to a real separation; according to the strength of the alcohol-water mixture used, arabans with a relatively low molecular weight are more easily removed than arabans with a higher degree of polymerization.

Moreover, the purity of pectic substances is dependent on the extraction conditions or on treatments after extraction, because the sensitivity to hydrolysis will vary. Arabans are more likely to hydrolyse than the pectinic acids. For this reason the amount of impurities mixed with the pectic substances as well as the amount of organic materials chemically bound to the polygalacturonic acid chains can be affected by the extraction procedure. By applying an extraction with 1.5 N HCl at 0–3°C during a long time, JAKOVLIV (230) has obtained from apple tissue pectins with a very small content of non-uronide materials (0.5 %) whereas other manners of extraction produced a pectin with a non-uronide content ranging from 5.5–9.7 %.

The results of several experiments indicate that non-uronide constituents are covalently linked to the polygalacturonic acid chains. JANSEN, MCDONNELL and WARD (234), PALMER and HARTZOG (369) and LINEWEAVER and JANSEN (285) concluded respectively from experiments on methanolysis, X-ray analysis and enzyme activity that some of these components are present in the chains, whereas HILLS and SPEISER (211), ANYASZ-WEISZ, SOLMS and DEUEL (10), NEWBOLD and JOSLYN (351) and other authors have pointed out that these constituents are attached as side-chains. According to LÜDTKE and FELSER (289) and SPEISER, EDDY and HILLS (450) arabinose and galactans are respectively bound to the pectic substances from flax and apples. As reported before, MCCREADY and GEE (329) have found arabinose, galactose, rhamnose and xylose in purified pectic substances from various sources.

As shown by NEUKOM ET AL (350) and HERI ET AL (206) the fractionation of sugar-

beet pectic acid on basic cellulose derivates has produced considerable evidence that araban and galactan are covalently linked to the polygalacturonic chains. These side-chains can be considered as a remainder of protopectin structure (368).

The presence of *acetic acid* in pectic substances was already claimed by EHRLICH and SOMMERFELD (153). From the work of VOLLMERT (496) and HENGLEIN (201) it can be concluded that a rather high percentage (6%) of acetyl groups can be found in sugar-beet pectins, whereas the acetyl contents in apple (0.3–0.4%) and citrus pectins is low. MCCREADY and MCCOMB (326) showed the presence of acetyl groups in pectic substances from peaches and pears.

Proof of the occurrence of *phosphoric acid* in ester or ionic linkage is claimed by HENGLEIN, KRÄSSIG and STEINMIG (203). While part of the P_2O_5 in the ash can be ascribed to inorganic phosphorus, a portion may be attached to the uronide chains and may play a part in protopectin structure.

A final remark has to be made on traces of ash constituents, especially aluminum, iron, and silicon which could not be removed from pectic substances by electrodialysis as was shown by EMMETT (155) and SPENCER (452).

From the discussion on structure it will be clear that pectic substances may show a very great *heterogeneity*, which is the result of variations in molecular weight, amount and distribution of methoxyl and acetyl groups and quantity and distribution of other non-uronide materials attached to the polygalacturonic chains as well as irregularities in these chains. From this nearly unlimited number of possibilities it cannot be expected that identical molecules are occurring in the same sample.

In addition to these variations in composition it has to be remembered that the shape of chain molecules in solution is not fixed as a result of the rotation of single bonds of the type C-C or C-O. Factors which restrict this rotation usually cause a stretching of the molecule. Such factors may be uncoiling in a streaming solution, solvatation and electrostatic repulsion between dissociated groups of the same macromolecule. DEUEL and NEUKOM (110) postulated that stretching of chains may be also the result of the introduction of side groups.

The hypothetical structure of protopectin

Protopectin has been defined as the water-insoluble parent pectic substances in plants from which soluble pectic substances can be produced.

In most plant tissues, the pectic substances are present in this insoluble form. The main exceptions are ripe fruits from which, after grinding and pressing or extraction with water, an important part of the pectic substances can be found in the juice or extract.

As compared with other tissues parenchymous tissue, forming the edible part of plants, and young meristematic tissue are known to be relatively high in content of pectic substances. The protopectin is most abundant in the intercellular layers and primary cell walls, while in secondary layers smaller amounts are present (see Fig. 5).

The insoluble pectic substances in plants are mixed with hemicelluloses. Further it has to be borne in mind that, very soon after cell division, cellulose and protopectin are deposited during formation of the primary wall. It is to be expected that during

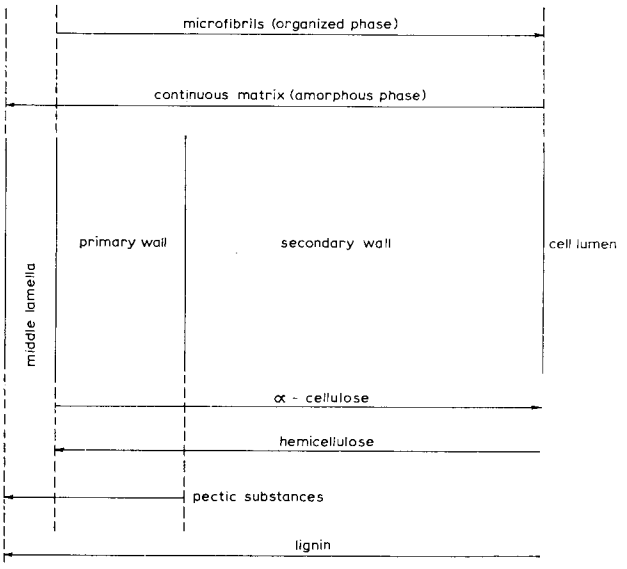


FIG. 5.
Distribution of materials in the mature cell wall. The arrows indicate direction of increasing relative concentration of the particular component. (Northcote, 352).

further development of tissues some cellulose is mixed with the constituents of the intercellular layer (171).

The presence of cellulose may be masked by protopectin (7, 169, 344, 520) and often cannot be detected by chemical microassay. In electron microscopy, the cellulose structure of the primary wall becomes visible only after removal of the protopectin by the use of pectolytic enzymes (520). The presence of cellulose in the primary wall is shown by the occurrence of a weak birefringrence (168), but this relation may be disrupted due to the fact that, in the inner parts of the primary wall, cellulose fibrils are not oriented in one direction (344, 520).

It has to be remembered that the cellulose fibrils are separated by hydrophilic colloidal material (as shown in Fig. 6) which consists largely of protopectin and hemicelluloses (7, 256).

The location of the greater part of protopectin in the intercellular layer and primary cell wall has given rise to the idea that the behaviour of pectic substances, *in situ*, may have an important influence upon the connection between the cells, in living as well as dead tissues. For this reason, the relation between this behaviour of pectic substances and the firmness of plants and plant products has been the subject of considerable study. However, it has to be stressed that changes in protopectin, (which is so intimately mixed with other cell wall constituents) may arise from alterations in the pectic substances as well from changes in the physical or chemical state of these other cell wall components. It has been shown by JERMYN and ISHERWOOD (235) that during ripening of pears the cell walls appear to be in dynamic equilibrium with cytoplasm and polysaccharides, especially arabans and xylans which are both broken down and synthesized.

It has already been mentioned that the structure of protopectin is unknown. Nevertheless a number of theories on protopectin structure has been developed, which have been derived mainly from extraction data. A compilation of these theories has been

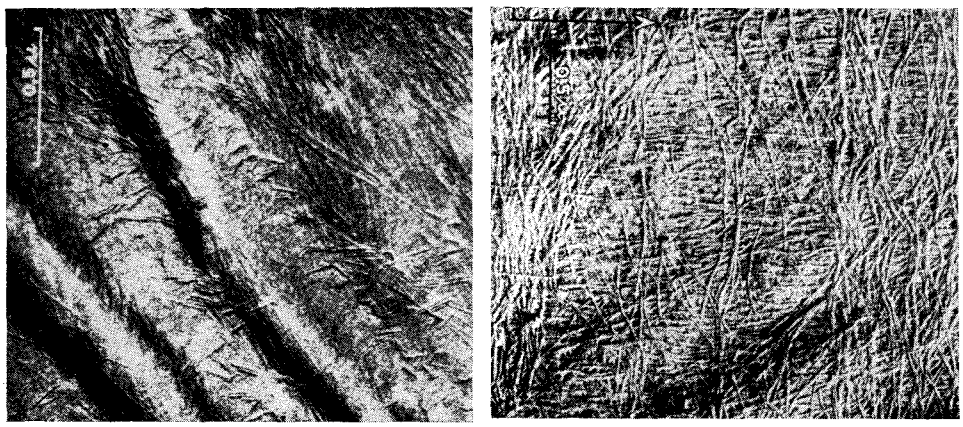


FIG. 6. Inside of cell wall of parenchymous cell from beet. Left: Cellulose microfibrils in amorphous interfibrillar material. Right: Cellulose fibrils visible after removal of interfibrillar material (Roelofsen, 407).

given by PALLMANN, WEBER and DEUEL (366) in 1944, which has been quoted by JOSLYN in his extensive review of chemistry of protopectin (245) in 1962. It is striking that during this eighteen years period no real new light has been thrown on this subject.

The possibilities of binding and anchoring of pectic substances in insoluble protopectin mentioned by PALLMANN ET AL (366) and reviewed by JOSLYN (245) are summed up in the following points:

1. Mechanical enmeshing or matting of the filamentous macromolecules of pectins by one another.
2. Mechanical enmeshing of the pectin molecule with other high polymers (cellulose, hemicellulose, lignin) of the cell wall.
3. Ester bond formation between the carboxyls of pectin and the alcoholic hydroxyls of other cell-wall constituents (cellulose, hemicelluloses, lignin).
4. Lactone bond formation within the entangled pectin molecule.
5. Salt bonding between the carboxyls of pectic substances and basic groups of proteins.
6. Polyvalent ion bonding (Mg, Ca, Fe) between the carboxyls of the entangled pectin molecules or between different primary valence chains of pectin.
7. Secondary valence binding (loose sorption, H-bonds, hydration bonding, molecular cohesion, etc.) between pectic substances or with other cell wall constituents.

Protopectin is considered to be an amorphous substance and optically isotropic. By means of polarization microscopy, X-ray diffraction and electron microscopy, ROELOFSEN and KREGER (408, 409) proved that the pectic substances in fresh collenchema cell walls of *Petasites vulgares* petioles consist of axially oriented submicroscopic fibrils, containing axially oriented crystallites. However, as far as we know, no indications have been presented on the occurrence of oriented pectic substances in other plant materials.

When pectinic acids are degraded they become increasingly soluble in water. In addition to the earlier mentioned possibilities of formation of insoluble protopectin KERTESZ (259) has postulated that protopectin may be considered to consist of very large molecules, which upon partial degradation become noticeably soluble in water. Further, KERTESZ has pointed out that the consistent presence of calcium in water-insoluble protopectins may indicate that this ion or perhaps other polyvalent ions also participate in the linking of polygalacturonic acids. This idea of calcium linkages in protopectin was developed further by HENGLEIN (199), HENGLEIN, KRÄSSIG and STEINMICH (203) and BOCK (54), who postulated that protopectin is formed by the association of polygalacturonic chains among each other and perhaps with cellulose and hemicellulose through calcium bridges between carboxyl groups. According to KERTESZ (259) 'the view expressed by HENGLEIN and BOCK is the first ray of light in a long time in the sterile field of protopectin chemistry'.

In contrast with pectic acid and pectinic acids with a relatively low degree of esterification, the pectinic acids with a higher degree of esterification ($> \pm 50\%$) are not made insoluble by the presence of calcium salts. There is no proof of the occurrence of native pectic substances with a high degree of esterification combined with such a high degree of polymerization that these substances are made insoluble by calcium ions, as has been suggested by KERTESZ (259). Up till now the highest degree of esterification of calcium precipitable high polymer pectinic acids has shown to be 50–60% (187); these results pertain to pectinic acids obtained by partial enzymic deesterification which is known to increase calcium sensitiviness of pectinic acids. However, artificial preparations of pectinic acids with about 10% free carboxyl groups, 30% of carboxyl groups esterified with methanol and 60% amidated carboxyl groups (see page 35), show coagulation and jellification in the presence of calcium salts. The tendency to form gels is increased with amidation (446, 447).

Nevertheless, from extraction data it is clear that the presence of calcium and other polyvalent ions in protopectin is exerting some influence on the insolubility of pectic substances, even when these possess a high degree of esterification. BAKER and WOODMANSEE (24) and MCCREADY ET AL (319) showed that the hot extraction of such pectic substances is enhanced by the use of sequestering agents, such as polyphosphates, whereas DOESBURG (140) found a beneficial effect from addition of ammonium oxalate during extraction of apple pomace at pH 3.3 and 80 °C. According to JOSLYN (245) Versene at pH 6 and room temperature did not extract more pectic substances from apple tissue than water alone. However, DOESBURG (131) showed that under these conditions the prolonged extraction was increased by addition of Versene as well as ammonium oxalate (see also Table 2). From these and other similar results it may be concluded that removal of calcium is important in relation to extraction of pectic substances in protopectin. DEUEL ET AL (112) stressed that this effect of calcium in protopectin is not to be attributed to the presence of calcium bridges between carboxyl groups of neighbouring filamentous molecules.

DEUEL, HUBER and ANYAS-WEISZ (112) have based their conclusion upon experimental data and theoretical considerations. Complete precipitation of a 50 % esterified pectinic acid required 4–5 times the quantity of calcium calculated from the free carboxyl groups (187).

Another indication of the absence of calcium bridges may be found in some of the jellying pheno-

mena of high-methoxyl pectins, which are also influenced by the calcium content of gels as will be described later; it is striking that the presence of calcium does not cause a real strengthening of these gels as might be expected by formation of calcium bridges.

We have to take into account that these negatively charged polyelectrolytes are surrounded by a cloud of cations which is in electrostatical equilibrium with the polyelectrical anion. The negative charge of the dissociated pectinic acids is counterbalanced by cations of added salts, which results in a decrease of ζ-potential. At the same salt concentrations this decrease of ζ-potential takes place to a greater extent the higher the valency of the cation of the salt present. According to HARVEY (194) the special properties of polyelectrolytes therefore become of particular importance whenever such multivalent metal cations are present. In gel formation by pectins the influence of metal ions is effective only when ionised carboxyl groups are present (194).

In the case of hydrophilic colloïds reduction of ζ-potential only is mostly not sufficient to cause flocculation, which may be performed by dehydrating agents such as ethanol, acetone or high salt concentrations. Therefore it is likely that also other factors are involved in the different behaviour of low-esterified and high-esterified pectinic acids to addition of calcium salts. In relation to swelling and precipitation differences in configuration (stretching of chains) have to be considered also as has been put forward by DEUEL and HUBER (111) and DEUEL and SOLMS (114).

To give an interpretation of the insolubility of pectic substances it is not essential to establish if calcium bridges between carboxyl groups are occurring or not. It may be sufficient to know that, by contrast with pectic acid and low-methoxyl pectinic acids, the high-methoxyl pectinic acids, abundantly occurring in nature, are not made insoluble by calcium. Therefore, other factors must be involved in the insolubility of these high-methoxyl pectic substances in protopectin. Such factors as covalent linkages, especially to hemicelluloses, have already been mentioned, and in addition, the secondary bonds (hydrogen bonds, etc.) to other cell wall constituents may be involved.

The suppression of the degree of swelling, as influenced by calcium and other polyvalent cations, may increase the tendency to form secondary valence bonds.

Apart from the primary and secondary bondings mentioned above, attention has to be paid to the mechanical enmeshing or matting of filamentous macromolecules of pectic substances with other high polymers of the cell wall as has been reported by TUTIN (480), MCCREADY and MCCOMB (326) and PALLMAN ET AL (366). However, it is not easy to show to which degree mechanical enmeshing is contributing to insolubility of protopectin, since most extraction procedures that have been tested usually lead to some depolymerization of pectic substances and other cell wall components. From some tissues, especially citrus albedo, pectins can be removed rather easily by washing, which may be an indication that mechanical enmeshing is an important factor. According to PILNIK (377a) the extent of disintegration of citrus peel during extraction is proportional to the pectin yield.

To exclude denaturation during extraction as far as possible, DOESBURG (131) has extracted, under very mild conditions, pectic substances from unripe apple tissue. The apple tissue was suspended and continuously stirred in solutions of 0.1 % acetic acid, 0.1 % acetic acid with 0.1 % oxalic acid or 0,1 % acetic acid with 0.5 % Versene. The pH of the suspensions was maintained at 6 by addition of a few drops of 25 % aqueous NH_3 and the suspensions preserved by keeping them under a layer of toluene. At the same time, a pectin solution with a content of 0.45 % of pectin and degree of esterification of 74.2 % was treated under the same conditions. The amount of solubilized pectic substances (expressed as the percentage of the total amount) from the

apple tissue was estimated after different times and the degree of esterification and molecular weight of the pectin were determined at corresponding periods. The results are shown in Table 2.

TABLE 2. *Treatment of apple powder solution and pectin solutions with acetic acid with addition of oxalic acid or Versene* (131)

Time	Apple powder suspensions						Pectin solutions			
	Acetic acid		Acetic and oxalic acids		Acetic acid and Versene		Acetic acid		Acetic acid and Versene	
	% soluble pectin	Esterification %	% soluble pectin	Esterification %	% soluble pectin	Esterification %	Mol. wt./10^3	Esterification %	Mol. wt./10^3	Esterification %
0 days	0.0	%	—	—	—	—	165	74.2	—	—
1 day	4.1	—	—	—	—	—	—	—	—	—
3 weeks	6.3	91.8	19.3	79.7	82.1	75.0	160	74.4	160	72.7
6 weeks	18.3	80.8	32.0	80.4	—	—	162	73.6	160	73.5

It can be seen that the molecular weight and the degree of esterification of a pectin in a solution are not affected for a long time at pH 6 in the presence of acetic acid or Versene. It is seen also that under these conditions the solubilization of a part of pectic substances from apple tissue takes place and that it is promoted by agents such as oxalic acid or Versene. It may be supposed that during the treatment of apple tissue with oxalic acid or Versene the pectic compounds swell rapidly, but the final loosening of pectic substances from the finely divided tissue needs a long time, presumably because it is retarded by mechanical enmeshing. This may be the reason that JOSLYN and DEUEL (250) did not find any appreciable increase of extracted pectic substances during treatment of apple tissue at room temperature at pH 6 after addition of Versene or ammonium oxalate. Unfortunately JOSLYN (245) has given no indication of the extraction time used. Similar results have been found by DOESBURG (131) when applying relatively short (3 days) treatment with ammonium oxalate to apple tissue. During this treatment, indications were obtained that the firmness of the apple tissue was decreased by swelling of pectic substances.

In these experiments (131), 1 cm cubes from peeled and cored apples (killed by keeping them for half an hour in the vapour of boiling ether) were suspended in solutions of 0.2 % acetic acid or 0.2 % acetic acid and 0.2 % oxalic acid. The pH of the suspensions of different lots of tissue cubes was adjusted to values ranging from 3 – 6.5 by addition of some drops of HCl or aqueous NH_3. The pH-equilibrium was reached after about 24 hours; 48 hours after this moment the amount of solubilized pectic substances, expressed as the percentage of the total amount in the apple tissue, was estimated. The firmness of tissue was measured by determination with the I.B.V.T.-hardness meter (135, 141).

As compared with suspension in the acetate buffers there was only a slight increase in the amount of soluble pectic substances by soaking the tissue in acetate-oxalate buffers. However, as compared with acetate buffer, suspension in oxalate buffers caused a considerable decrease of firmness of tissue, which may be an indication of swelling of pectic substances in the intercellular layers. These changes in firmness of apple tissue correspond fairly well with the influence of acetate buffers or acetate and oxalate buffers on firmness of pectin films as will be reported later (Chapter 2).

The results of these trials are summarized in Fig. 7.

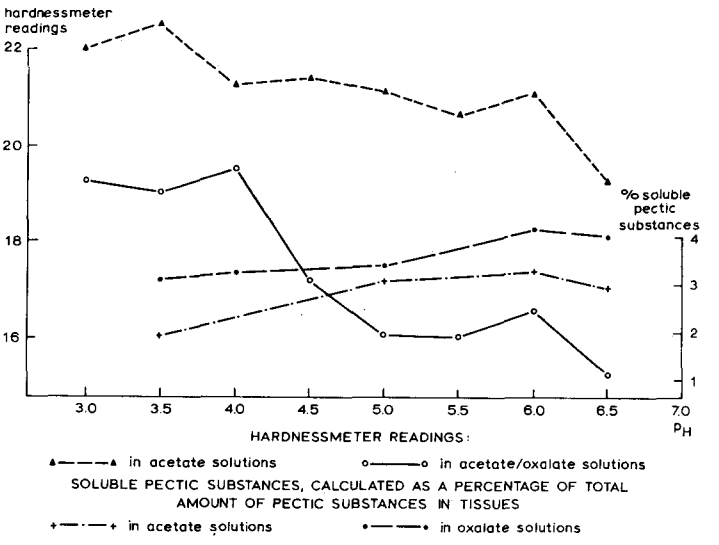


FIG. 7.
Influence of pH, acetates and acetate-oxalate mixtures on firmness of apple tissue, (Doesburg, 131 and 137).

More evidence of the possibility of mechanical enmeshing of pectin molecules may be derived from the communication of PILNIK to JOSLYN (245) that the pectin content of liquid expressed from acid-treated apple pomace decreases with increase of pressure. The liquid first expressed is high in pectin content, which decreases to less than one-third of its initial concentration at the later stages of pressing, when the compression of the pomace is highest. It has to be assumed that during filtration of the pectin-containing liquid through the strongly compressed pomace the movement of pectins is prevented. Similar effects have been found by DOESBURG (140) when filtering crude extracts form apple pomace, containing charcoal for decolorization. The filtrate obtained in the latest stages of filtration with the use of a relatively high pressure contained a considerably lower pectin content than the first 90% of the total amount of filtrate obtained.

The degree of mechanical interlacing of the filamentous pectic macromolecules with one another or with high polymers of the cell wall and the occurrence of secondary bonds is influenced by pre- treatment of plant tissue before extraction, especially by the higher temperatures used in commercial dehydration.

The effect of extraction procedures depends on the possibility of diffusion of macromolecules out of the cell walls as well as the possibility of penetration of solvents or extractants. For this reason it is clear that the manner of pre-treatment of raw materials before pectic extraction should be of great interest and importance.

Recently, JOSLYN and DEUEL (250) reported on the marked differences they found in extractibility in marcs prepared in various ways from the same lot of apples. Special attention has to be paid to the fact that enzymatic browning during preparation decreased the solubility of pectic substances, which shows that insolubility can be caused also by other factors than the structure of protopectin itself. This subject will be discussed further when dealing with apple pomace (Chapter 5).

As shown by SHIOIRI and HAGINUMA (431) the yield of pectins, extracted at 100°C

from apple pomace, is increased by addition of salts such as sodium chloride and sodium sulphate. This may be another indication that the binding of pectic substances in the tissue is affected by the physical state of the cell wall constituents.

Summarizing the foregoing discussion it can be stated that the insolubility of protopectin may be caused by:

1. Covalent bonding of pectic substances to other cell wall constituents, especially hemicelluloses and association with other cell wall components by secondary bonds.
2. The presence of cations, especially Ca^{2+}, leading to insolubility of low-esterified pectic substances and reduction of swelling of the higher esterified pectic substances.
3. Mechanical enmeshing of filamentous macromolecules of pectic substances one with another or with other polymers in the cell wall.

Chapter 2

Some important characteristics of pectic substances

Coagulation

Polysaccharides and other water-soluble polymers may be coagulated by addition of organic compounds and inorganic substances, especially electrolytes.

The flocculation of such hydrophilic polymers is governed by many factors (114):

1. The constitution of the organic compound added.
2. The valency of electrolytes (SCHULZE-HARDY and HOFMEISTER series).
3. The presence, distribution and number of dissociated groups (equivalent weight) as well as the character of these groups and their ability to form insoluble complexes.
4. The degree of polymerization of these polymers.
5. The branching or non-branching of these polymers.
6. The presence of side-groups which are masking functional groups which together with dissociated groups influence the kinking of polymer chains.
7. The concentration of polymers.

It is not easy to identify the causes of differing susceptibility to coagulation of various polymers. The flocculation of polysaccharides and other polymers by electrolytes has been investigated by DEUEL and SOLMS (114). They found that sensitiveness to coagulation is strongly diminished by decreasing concentration and decreasing number of dissociated groups. Many differences in the behaviour of various polysaccharides with similar constitution could not be elucidated and had to be attributed to unknown differences of molecular weight and kinking or branching of molecules.

It is known that susceptibility of pectinic acids to coagulation increases with rise of molecular weight and decrease of degree of esterification.

As reported by HAAS-SCHULZ (187), apple pectins partly deesterified by tomato pectin methylesterase to a degree of esterification of 60%, can be coagulated by calcium when their average molecular weight is not below 14.800. However, it needs to be postulated that, in enzymatic saponification, the esterified carboxyl groups are not attached randomly, as will be described later (Fig. 41). This phenomenon causes a greater heterogeneity of distribution of free carboxyl groups along the molecular chains which results in a somewhat greater sensitiveness to coagulation by calcium.

According to DEUEL ET AL (112) pectinic acids are not precipitated by calcium when their degree of esterification is greater than 50%. Similar results have been obtained by HILLS, WHITE and BAKER (209).

As has been mentioned before, precipitability by calcium is of special interest in relation to discussions on protopectin structure, because in some products the insolubility of low-methoxyl pectinic acids (caused by calcium salts) has led to the firming of plant tissues by addition of these salts. Since pectic substances act as cation exchan-

ge resins, the influence of other cations should also be taken into account, especially whenever such cations are present in greater quantities. The beneficial effect of addition of salts such as sodium chloride and sodium sulphate on pectin extraction (431) is already mentioned in Chapter I.

When taking into account the relative amounts present in plants it does not seem likely that other polyvalent cations are important in relation to insolubility of pectinic acids in plant tissues, since the numbers of milli-equivalents required for coagulation of the same amounts of 0.5% sodium pectate in water were found to be 2 meq. $CaCl_2$, 1.20 meq. $FeCl_3$ and 20 meq. $MgCl_2$ (114).

As reported by JOSLYN (245), precipitation with calcium, lead or copper salts may be used to recover low-methoxyl pectins from water solutions, whereas the application of aluminum chloride or sulphate is very often used for the precipitation of high-methoxyl pectins with a degree of esterification up to 80%. From the results of MCCREADY ET AL (323) it can be concluded that pectins with a degree of esterification of 60% or even higher can be precipitated by addition of cupric sulphate.

TIBENSKY, ROSIK and ZITKO (476) stated that pectinic acids with a degree of 90% or higher can be precipitated with cupric ions.

The yellowish-green precipitates obtained after addition of aluminum salts may be regarded as a co-precipitate of negatively charged pectinic acids and positively charged aluminum hydroxide.

In commercial pectin production, the aluminum chloride or sulphate solution is added to pectin-containing extracts in amounts indicated by laboratory checks for each particular tank of liquor. Sodium carbonate or ammonium hydroxide is added until a pH 3.8 to 4.2 is reached; according to JOSEPH and HAVIGHORST (244) most batches exhibit optimum precipitation near pH 4.0. With this increase of pH, the aluminum salt hydrolyses and forms colloidal aluminum hydroxide in the pectin-containing extract (see Fig. 8). After filtering and pressing the precipitate, it is washed in acidified ethanol to effect the removal of aluminum (244).

Pectic acid and pectinic acids with a low degree of esterification can be precipitated by addition of acids. The precipitation and isolation of pectic acid has been used as a method for determination of pectic substances (351, 516). The acidic isolation of low-ester pectins has been studied by MCCREADY ET AL (322). They showed that it may be used in the production of these pectins (363).

Low-methoxyl pectins deesterified by alkali or acid, can be precipitated in yields greater than 90% by use of acid solutions below pH 2 at temperatures below 25°C, when the methoxyl content of high polymer pectins is below 4%; enzymatically deesterified materials can be obtained in 80% yield or above at methoxyl contents less than 7%. Thus, enzymatically deesterified pectins are more sensitive to precipitation.

The coagulation of pectic substances from aqueous solutions by addition of ethanol or acetone is widely practised.

The use of acetone produces a more firm and filamentous coagulum than the same amount of ethanol. For this reason HINTON (213) prefers acetone. However, when comparing additions of the same amounts of both precipitants it has been found that a greater amount of non-uronide materials will be enclosed in the precipitates pro-

FIG. 8. Solutions of aluminum chloride and sodium carbonate are added to adjust pH for precipitation of 'green pectin' (Joseph and Havighorst, 244).

duced by addition of acetone. For purification purposes the final acetone contents should not exceed 50% (v/v), whereas ethanol may be used to 70% (v/v).

In relation to the degree of polymerization, partial fractionation of pectic substances may be accomplished by the addition of different quantities of ethanol or acetone. Pectinic acids with a low degree of polymerization are not flocculated by lower concentrations of these precipitants, whereby the quantity of enclosed non-uronide matter may be lower. This effect has been demonstrated by PEYNAUD (375) in the purification of pectinic acids, with extracts from peaches. Thus, to precipitate the total amount of pectic substances in these extracts an addition of at least 63% ethanol was shown to be necessary.

Special attention has to be paid to the flocculation of pectinic acids by other polymers, especially polymer bases. Optimal flocculation may be expected when the total negative charge of the polymeric anions corresponds to the positive charge of the polymeric cations. Since the dissociation and charge of weak polyelectrolytes is influenced by the pH the conditions for optimal coagulation will be depending on pH also; the same holds for the influence of common electrolytes which are lowering the electrostatical charge.

Experiments on flocculation between pectinic acids and soluble polyamides have been described by DEUEL, SOLMS and DENZLER (115, 117). They found a nearly stoichiometric relationship between polyamide and pectinic acid:

$$\text{Pectinate}^- \ \text{Na}^+ + \text{Polyamide}^+ \ \text{Cl}^- \rightleftharpoons \textit{Pectinate}^- \ \textit{Polyamide}^+ + \text{Na}^+ \ \text{Cl}^-$$

With increase of degree of esterification of pectinic acid, a smaller amount of polyamide showed to be needed. It is not likely that one single polycation is neutralized by one polyanion, but it has to be expected that electrostatical equilibrium exists between a number of molecules of both polymers. These flocculation reactions may be used for clarification of fruit juices (117).

ZITKO, ROSIK and VASATKO (528) have reported on flocculation reactions between gelatin and pectinic acids, which may cause flocculation between pH 2.5 and 4.75 (= iso-electric point of gelatin). The ratio of weights of pectinic acid and gelatin needed to obtain optimal flocculation depends on pH conditions; a formula for this relationship is given. The flocculation of pectins, gelatin and tannins during the clarification of fruit juices is described by ZITKO and ROSIK (527).

According to DREWES (145) and ZIEGELMAYER (526) flocculation of casein occurs after addition of pectins to milk, even when the pH of the milk is not affected by this addition. JOSEPH (240) stated that the addition of 0.2–0.3 % pectin to fresh, pasteurized, skimmed or homogenized milk coagulates casein in 10–30 minutes. This reaction did not occur with evaporated, condensed, and reconstituted dried milk.

DOESBURG and DE VOS (142, 144), when using high-methoxyl powder pectin, found it possible to disperse and solubilize pectin preparations in milk during about 10 minutes of stirring. After the pectin is solubilized macroscopic coagulation of a casein-pectin complex can be prevented by addition of fruit juice or an acid solution under continuous stirring till a pH $\pm$4.2 to 3.0 is reached. Then the whole mixture can be preserved by pasteurization without curdling. It can be seen from Fig. 9 that some microscopic coagulation takes place during solubilization of pectin, but even after pasteurization the coagulation has not increased.

According to this method, pasteurized mixtures of fruit juices and milk with a long shelf life can be produced. In spite of the fact that particles of microscopical size are formed the beverage is not gritty at all.

When returning to factors influencing coagulation it is not easy to explain why coagulation is inhibited by acid addition when this normally causes curdling of milk proteins. It was mentioned before that in mixtures of milk and pectins at pH $\pm$ 6.5 after some time a heavy precipitate is formed. It may be that this affinity of pectin and milk proteins has a protective influence on the proteins during the acidification of the milk when the pH is passing the iso-electrical point of the proteins.

When milk is acidified previously to pectin addition, it will be impossible to yield a good result, even when the protein precipitate has been dispersed again by homogenization. From these results it may be concluded that the action of pectin during acidification is very important; nevertheless it has to be supposed also that pectin is acting as a stabilizing agent during shelf life. In contrast to the upper limit of pH ($\pm$ 4.2) no sharp lowest limit could be found. The sharp upper limit of pH may be explained in two manners: a) the stability of milk proteins in the neighbourhood of their iso-electrical point is too small to resist pasteurization, b) it has been shown that the degree of polymerisation of pectins is strongly diminished during heating at pH values higher than $\pm$4, which will cause also a strong decrease of the stabilizing value of pectins during heat treatment and storage.

With low-methoxyl pectins no similar results could be obtained.

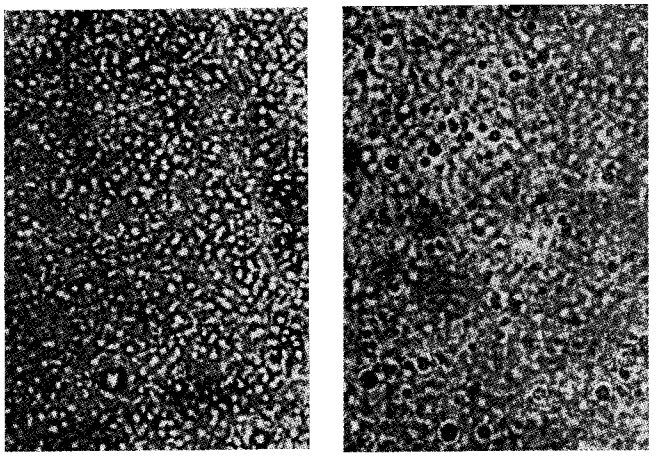


FIG. 9.
Microscopical photos of mixtures of milk, pectin and fruit juice. Left: before pasteurization. Right: after pasteurization. (Doesburg and de Vos, 142).

Swelling

A strong relationship exists between the solubility and swelling ability of hydrophilic colloids. In many cases swelling may proceed to solubilization which is shown in Fig. 10.

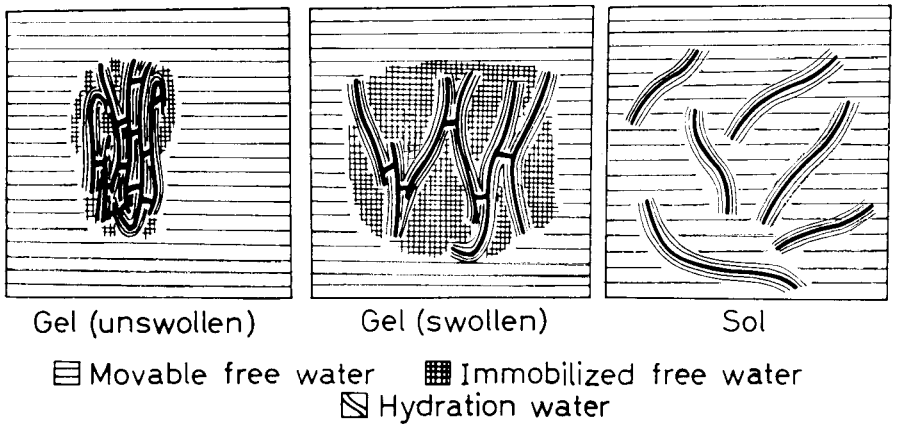


FIG. 10. Influence of cross-linking on swelling of a macromolecular system (Hamm, 190).

Protopectin and covalently linked pectinic acids show a limited swelling ability in water. The degree of swelling is dependent on the structure of the network, the molecular weight, degree of esterification, the presence of side-chains, pH and the presence of salts in the surrounding medium.

The swelling ability of pectic substances is enlarged by increase of molecular weight and degree of esterification. When studying the swelling properties of polygalacturonic acids partly esterified with oxypropyl DEUEL and HUBER (111) and DEUEL, HUBER and ANYAS-WEISZ (112) found that the swelling ability was promoted by increase of the degree of esterification. According to HENGLEIN and KROHN (204) and HOOGZAND and DOESBURG (223) the swelling power of dead vegetable tissue is decreased with diminishing degree of esterification and polymerization of the pectic substances in these tissues as shown in Fig. 11.

The swelling power of pectic substances in acid media is enlarged with rise of pH.

Insoluble pectic substances can be regarded as cation exchangers. Differences of swelling caused by various cations are influenced by the position of these cations in the lyotropic range. Mostly the influence on exchange of cations as exerted by the anions in the surrounding solution is negligible, except when these anions are forming complexes with some of the exchangeable cations.

Ca^{2+} —Na^{+} exchange equilibria between artificially cross-linked pectic acid (linked with formaldehyde) as cation exchangers and external solutions with different anions have been measured by DEUEL ET AL (118). They showed that at pH 4.2–7.4 the Ca^{2+} selectivity of these exchangers is decreased by the used anions in the following order: acetate, β-indolyl acetate, citrate, ethylenediamine tetra-acetate. The swelling of cross-linked pectic acid increases with increase of sequestering ability of the anions for

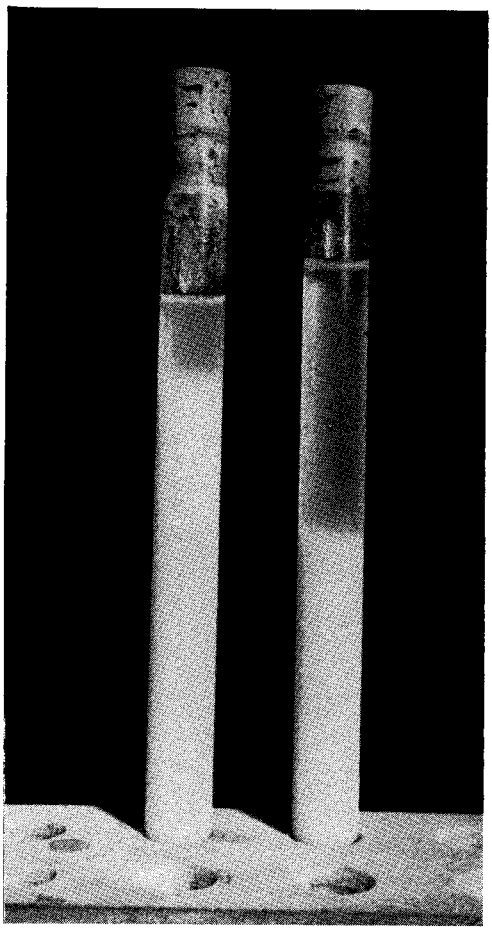


FIG. 11. Swelling of powder, made from cauliflower after application of different blanching conditions (1 g powder in 20 ml water). Left: tissue particles containing pectic substances of a higher degree of esterification. Right: particles containing pectic substances with a lower degree of esterification. (Hoogzand and Doesburg, 223).

Ca^{2+} ions. The relationship between swelling of pectin films in 35 % ethanol, as reported by DOESBURG (131, 137), is shown in Fig. 12.

The films have been made by drying a solution of purified pectin on glass plates; the pectinic acid content of these films was 96 %, the degree of esterification 75 %, their calcium content 0.78 %. The film was cut into rectangular 7 x 7 cm pieces, each weighing about 4.8 g. Two 35 % ethanol solutions containing 0.2 % acetic acid or 0.2 % acetic acid and 0.2 % oxalic acid were divided into 8 aliquots of 250 ml each. The aliquots were adjusted with NH_3 solution to various pH-values, ranging from 3.0 to 6.5.

Four rectangular pieces of the pectin film were put into each aliquot for 24 hours, while the original pH-values of these aliquots were maintained by addition of HCl or NH_3 solution.

After this treatment, the strength of the pectin films was determined by measuring the force to penetrate them with the metal pin of the I.B.V.T. hardness meter (135, 141) as shown in Fig. 13. The increase in weight was determined as an indication of swelling.

It is clear that the increase of swelling of the pectin film at elevated pH-values and in the presence of oxalates corresponds to a decrease of firmness. A comparison with

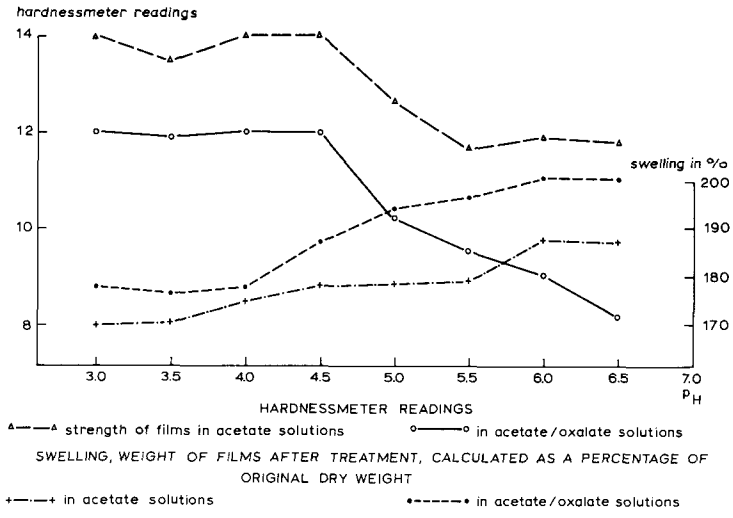


FIG. 12. Swelling and firmness of pectin films in acetate or acetate-oxalate solutions with different pH. The swelling is increased and the firmness decreased by removal of calcium and increase of pH. (Doesburg, 131, 137, 138).

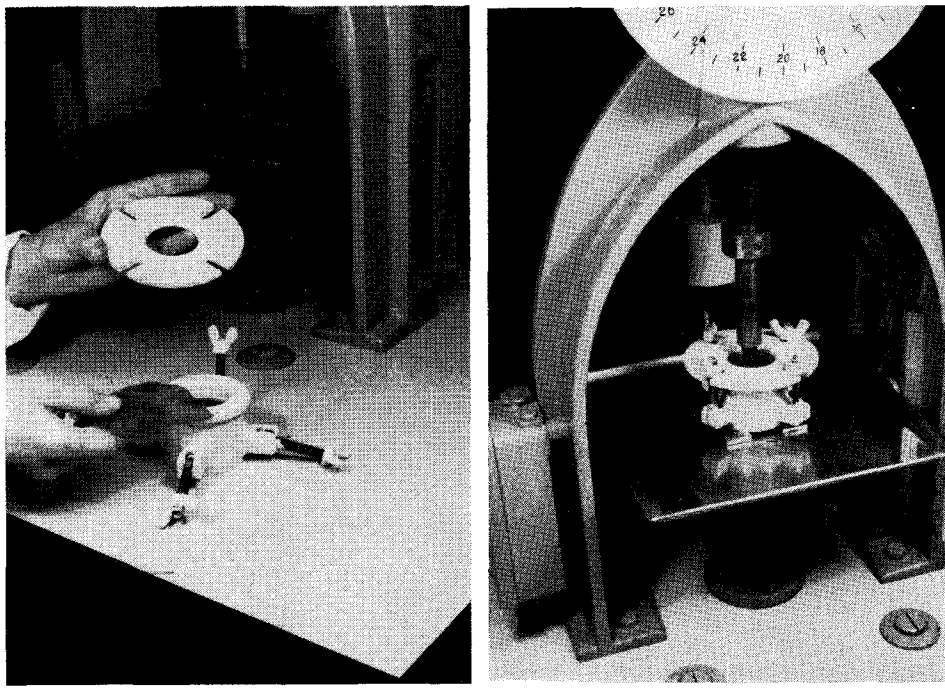


FIG. 13. Measurement of strength of pectin films with I.B.V.T.-hardnessmeter. Left: clenching of swollen pectin films between two metal rings. Right: measurement of strength by punching with metal pin (Doesburg ,131, 137, 138).

the corresponding results for addition of calcium binding acids and variations of pH to pectin films (Fig. 12) and to killed plant tissues (Fig. 7) makes it probable that the effect of these acids and the pH on firmness of plant tissues may be attributed also to an increase of swelling of protopectin. For this reason the firmness of cooked plant tissues may be expected, partly to depend on the character of the anions present.

The differences of firmness of plant tissues, which may be ascribed partly to differences of swelling properties of insoluble pectic substances in cell walls, can be measured easily. However it may be difficult to show the relationship between swelling properties of the insoluble pectic substances and the swelling of non-desintegrated tissues in which these are occurring. It has been possible to prove such a connection in powdered and washed alcohol-insoluble solids from cauliflower tissue (Fig. 11) but the swelling differences of non- disintegrated tissues may be expected to be small (131).

Solubilization

Pectins should be completely dissolved to exhibit their maximum jellying power or to yield maximum viscosity.

To a limited extent lumping can be avoided by seeding the pectin at a slow rate into water during continuous stirring. The dispersion and solubilization is promoted when the required amount of pectin is thoroughly mixed with five to eight times its weight of granulated sugar.

Experience has shown that pectins dissolve best in solutions containing no more than 25% soluble solids. To obtain a complete solubilization of pectins in jam or jelly manufacture it is advisable that the main part of the sugar is added after the pectin is dissolved during heating or boiling.

It is clear that the solubilization may be quick when the pectin shows a great dispersability which prevents lumping just after addition to water.

The dispersability of pectin particles can be promoted by 'coating' with a very small amount of suitable metal ions such as aluminum, copper, iron, nickel and chromium (294).

According to SCHILT (418) a different rate of solubilization is found for pectin preparations manufactured respectively by spray drying or dehydration of precipitated pectins. When studying the rate of solubilization of pectin suspensions under the microscope, spray dried pectins proved to dissolve more easily than the other pectins. During solubilization of spray dried pectins air bubbles are produced from air which is enclosed in the pectin particles. However, under practical conditions greater quantities of spray dried pectins show a lower rate of solubilization by lumping as a result of an increased floating tendency by enclosed air.

To effect the solubilization of a pectin preparation under laboratory conditions, it is very useful to wet it with some drops of ethanol or acetone prior to suspension in water. The wetted pectin may be easily suspended in water without lumping and the pectin is dissolved rapidly during stirring, when the ethanol or acetone is diluted by the surrounding water.

Since low-methoxyl pectins are coagulated by calcium, these pectins have to be solubilized in water with none or a low calcium content.

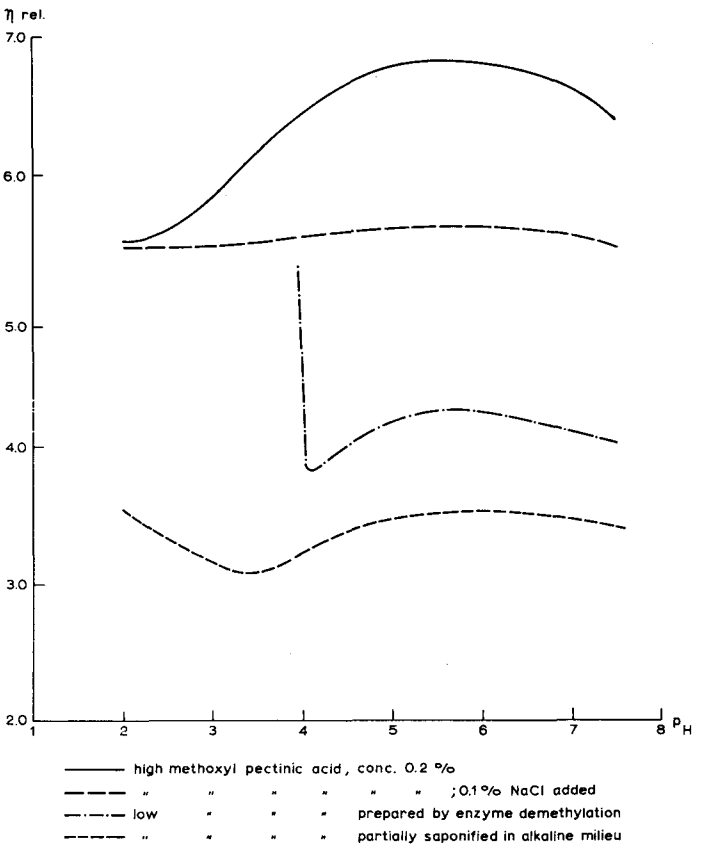


FIG. 14.
Relative viscosity of solutions of high-methoxyl pectin (with or without salt addition) and of low-methoxyl pectins, prepared by saponification in alkaline milieu or enzymic treatment (data of Schultz, Lotzkar, Owens and Maclay, 287, 426).

Viscosity

Solutions of pectic substances may show a high viscosity, which is important in the behaviour of various products (e.g. fruit juices and apple sauce).

The determination of viscosity of solutions of pectic substances has often been used to calculate their molecular weight, according to the principles given by STAUDINGER, and also to study the chemical and enzymatical breakdown of pectic substances.

The viscosity of a solution can be characterized by the related function of *specific viscosity*

$$= \eta_{sp} = \eta_{rel} - 1 = \frac{\eta - \eta_0}{\eta_0}$$

In this equation,

η = viscosity of the solution of pectic substances.

η_0 = viscosity of the solvent.

When using an Ostwald viscosimeter, the *relative viscosity*, has to be calculated from viscosimeter-time readings:

$$\eta_{rel} = \frac{t.sw}{t_0.sw_0}$$

t = time of flow of solution of pectic substances.
t_0 = time of flow of solvent.
sw = specific weight of solution of pectic substances.
sw_0 = specific weight of the solvent.

As rather diluted solutions (concentration $<1\,\%$) are used, the influence of specific weight may be neglected and η_{rel} is calculated from the ratio t/t_0.

The use of the *intrinsic viscosity*, $\eta_{sp/c}$, is often replaced by the ratio $\eta_{sp/c}.100 = Z$ ('Zähigkeitszahl'), in which c is the molar concentration of the monomer unit in the solution. In pectic acid solutions containing X g pectic acid per liter $c = X/176$, in solutions of fully methoxylated pectinic acids $c = X/190$.

According to DEUEL (106) the value of intrinsic viscosity found, can be used only in relation to the concentration of pectic substances in the solution from which the determination has been produced; generally extrapolation of intrinsic viscosity to zero concentration is not permissible for heteropolar colloids.

The intrinsic viscosity is increased with dilution of pectinic acid solutions and this is attributed to further dissociation of carboxyl groups. With rising concentration of pectinic acids the intrinsic viscosity exhibits a minimum, but increases again at still higher concentrations. The latter effect may be due to association of pectinic acid molecules.

Many experiments on factors governing the viscosity of solutions of pectates, pectinates or pectinic acids have been made; especially the work of DEUEL ET AL (106, 109, 118), LOTZKAR ET AL (287) and SCHULTZ ET AL (426) has to be mentioned.

The viscosity of water solutions of pectinic acids is dependent on molecular weight, degree of esterification, presence of electrolytes and pH, and concentration.

The higher the molecular weight, the greater the viscosity. Usually decrease of degree of esterification is accompanied by a drop of viscosity, however, when comparing the viscosity caused by high-methoxyl pectinic acids with their low-methoxyl derivates it is necessary to take into account that, during saponification, some breakdown of polygalacturonic chains may occur. Thus, the decrease of viscosity by partial deesterification as reported in many cases, may be caused partly by some depolymerization. When the degree of esterification is very low and results in a decrease of solubility, the viscosity is increased.

It has already been pointed out that the reaction products of enzymatical deesterification are more liable to flocculation by electrolytes or by lowering of pH than low-methoxyl pectinic acids produced by saponification in acid or alkaline environment. When comparing the relation between viscosity and pH of solution the increase of viscosity of enzymatic deesterified pectins by partial insolubility will be found at a higher pH-value than for alkali or acid treated low-methoxyl pectinic acid (Fig. 14).

For high-methoxyl pectinic acids, decrease of pH does not lead to insolubility. For this reason a strong increase of viscosity at low pH-values is not found and the viscosity is increased with dissociation of carboxyl groups at higher pH-values.

By addition of salt the maximum of viscosity at pH $\pm$ 6 can be suppressed as shown in Fig. 14. The magnitude of this depression of electroviscous effects increases with decrease in methoxyl content; as compared with high-methoxyl pectins increased

charge per unit weight of the low-methoxyl pectinic acids could account for this increase of depression.

According to LOTZKAR ET AL (287) the viscosity is raised in increasing order by the addition of salts of calcium, strontium, barium, aluminum, copper and nickel. The factors that are involved are reduction of ζ-potential and the formation of aggregates of various shapes, sizes and degrees of hydration.

Jellying phenomena

In Chapter I pectins have been designated as those water-soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels under suitable conditions.

Pectins are able to form stable, solid gels which are used in the food industry. These gels can be divided into two groups, those with a rather high sugar content ($> \pm 50\%$) and gels with a lower sugar content. For the first group high-methoxyl pectins (degree of esterification higher than $\pm 50\%$) are used, whereas low-methoxyl pectins are mostly applied in low-sugar gels.

The mechanism of jellification has been discussed by various authors (125, 193, 214, 357) on the basis of precipitation of pectin under the prevailing conditions of jelly formation, whereas a uniform dispersion of pectin molecules is maintained. In other words, gels are commonly regarded as two-phase systems with a high degree of interface between a continuous, or at least intermeshed, system of solid material holding an aqueous (or other solvent) phase which may also be continuous or finely dispersed (305).

As has been reported before (Fig. 2) pectins have a relatively high degree of polymerization. Since there is a rather strong relation between jellying power and degree of polymerization (43, 78, 143, 190, 259, 359) it is not surprising that in many cases also a strong relationship between jellying power and intrinsic viscosity has been found. OLSEN ET AL (359) reported that molecular weights of about 40,000, 100,000, and 175,000 respectively, correspond with poor, moderate, and very good jellying powers.

One of the main factors which disturb the relation between molecular weight and jellying power arises from the presence of side-chains or side-groups which are covering functional groups needed for jellification. The same factor has been mentioned in relation to coagulation of hydrophilic polymers.

According to DEUEL, SOLMS and ALTERMATT (116) components covalently linked to the polygalacturonic chains influence the jellying ability, which has been mentioned also by JONES (238). In some cases, especially beet pectins, the jellying power is dependent on the removal of such side-groups during extraction or other treatments.

As shown by NEUKOM (346) ethyl, oxyethyl, mono-oxypropyl and dioxypropyl esters of pectic acid possess no jellying properties in a milieu with high sugar content. VAJDA (489) stated also that oxypropyl ester of pectic acid has no jellying properties under these conditions. Further the influence of formation of amides has to be mentioned here; derivates containing more than 70 % of amidated carboxyl groups are insoluble in water. The tendency to form gels is increased with amidation (446, 447).

Of special interest is the influence of acetyl groups (6 %) in sugar-beet pectinic acids which prevents jellification of such pectinic acids (201, 346, 496). PIPPEN, MCCREADY and OWENS (380)

prepared a series of pectin acetates and found the ability of the pectin to form high-solids gels markedly reduced or eliminated when 2.6 % or more acetyl was present. By contrast with the action of relatively low amounts of acetyl groups present in beet pectinic acids, it has been found that pectic acid, strongly esterified with acetic acid, may show jellying ability in sugar and acid-containing milieu. Thus, the jellification is hindered by the presence of relatively low amounts of acetyl groups but with a more extensive acetylation a new surface of hydrophobe acetyl groups will be formed which is enabling gel formation (445).

BOCK (54) developed a special theory on pectin production from sugar-beets based upon the protopectin hypothesis of HENGLEIN (199). According to BOCK these pectins have to be extracted in rather strong acid environment at 30°–50°C, to avoid hydrolysis of polygalacturonic chains and to promote the lifting of interlocking ionic linkages (calcium bridges). From our present knowledge on constitution of sugar-beet pectinic acids it is clear that the success of this manner of extraction of beet pectins is based upon the removal of acetyl groups.

High-methoxyl pectins

When a sufficient amount of high-methoxyl pectin is present for the formation of a gel two other conditions have to be fulfilled:

1. the electro-static repulsion between pectin molecules has to be decreased by depression of dissociation of carboxyl groups.
2. sucrose or similar compounds (also alcohols or polyalcohols) have to be added in a sufficient amount. The lower limit of sugar content has been shown to be about 40%. In most cases, e.g. jam manufacture, the sugar content is considerably higher.

The *jellying power* of pectins is related to their sugar carrying power. It is customary to use the definition: *jelly grade* is the proportion of sugar which one part of solid pectin (or pectin extract) is capable turning, under prescribed conditions, into a jelly with suitable characteristics. For instance, from a pectin of 200 jelly grade in a gel with 65% sugar, a content of 65/200% of this pectin is needed to produce a standard gel. For the determination of jellying power the reader is referred to Chapter 3.

In Fig. 15 the relation between jellying power and pH in gels with a sugar content of 65% is shown for purified pectins with various degree of esterification.

All pectins show a *maximum pH for jellification.* When the pH is decreased below this maximum pH, the jellying power increases till a constant value is reached. This relation between jellying power and pH can be explained when taking into account the dissociation of carboxyl groups. By decrease of pH, the dissociation of carboxyl groups is depressed which reduces the electro-static repulsion between pectin molecules, thus enhancing the possibilities of regions of contact between these molecules. Finally, further decrease of pH does not cause a greater jelly strength, since within the scope of a homogeneous distribution of pectin molecules over the gel volume no more places of contact will be available when the dissociation is depressed further.

From Fig. 15 it is evident that the maximum pH at which jellification is occurring, is lowered by decrease of the degree of esterification of pectins. This upper limit of pH is raised by addition of some calcium or other polyvalent cations.

The maximum pH for jellification of a pectin is dependent also on the sugar content of the gel. An increase of sugar content is causing an increase of this maximum pH. In gels with a soluble solid content of $\pm$ 72%, containing a pectin with a degree of esterification of $\pm$ 80% the maximum pH showed to be $\pm$ pH 4.2, whereas at a sugar content of 65% the maximum pH is $\pm$ 3.7.

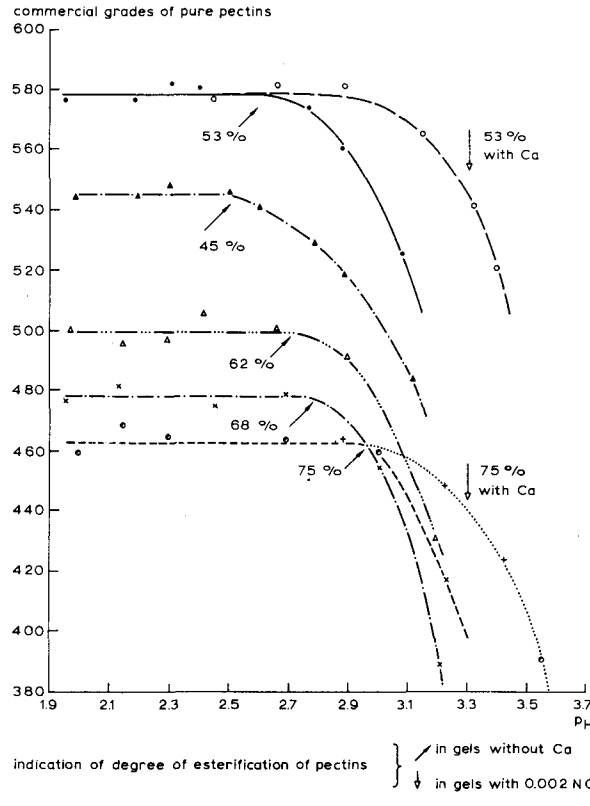


FIG. 15.
Relation between jellying power and pH in pectin-acid-sugar gels of apple pectins with different degree of esterification. Influence of calcium salts. (Doesburg and Grevers, 143).

The maximum gel strength is promoted by increasing sugar content (76). Hydrolysis of sucrose during boiling has no influence upon final gel strength which has been shown by COLE, COX and JOSEPH (83). The relation between gel strength, pH and sugar content as influenced by practical conditions of jam manufacture will be discussed later in relation to setting time phenomena.

The maximum jellying power of a pectin sample is enlarged by partial saponification under suitable conditions. This effect can be seen already from the data in Fig. 15; the pectins with a lower degree of esterification which are showing a greater jellying power are manufactured from the pectin with the highest degree of esterification (75%).

According to a recent publication of HAMM (190) the effect of sugars on jellification is not yet completely understood. The oldest and most simple theory is that sugars are removing the protecting hydration layer of pectin molecules, thus enabling these molecules to approach each other to form cross-linking bonds (e.g. hydrogen bonds between carboxyl groups and between other hydroxyl groups of neighbouring molecules). However other possibilities of the action of sugars and other dehydrating substances have to be taken into account also.

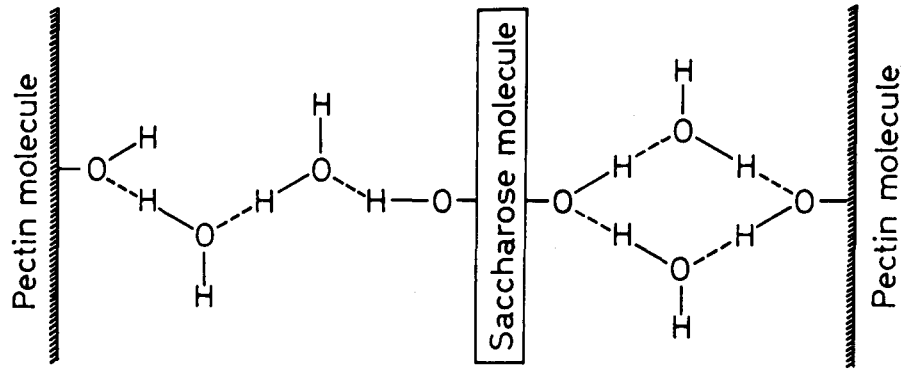


FIG. 16. Presumed structure of a pectin sugar gel (Henglein and Anders, 205 and Hamm, 190).

The possibility exists that jellification is connected with the formation of cross-linking hydrogen bonds between the hydroxyl groups of the sugar and pectin molecules, whereas HENGLEIN and ANDERS (205) have suggested an orientation of the water dipoles by the sugar molecules Fig. (16); the dipoles are attracting each other mutually and should cause a stiffening of the gel which should become more marked with increasing chain length of the pectin molecules.

The influence on jellification of various dehydrating agents has been discussed extensively by TÄUFEL and BERSCHNEIDER (473). They have pointed out that a close relation may not always be found between dehydrating power of various substances (as measured by their influence on swelling of potato starch) and their promoting influence on jellification. The effect of these substances should be depending also on their influence on dielectric constant. A decrease of dielectric constant should lead to a decrease of electrokinetic ζ-potential, which is known to promote aggregation of colloids.

According to the view of the author the effect of calcium ions, shown in Fig. 15, must also be explained from a decrease of ζ-potential, which is enhancing association of pectin molecules in that pH range, where dissociation of carboxyl groups is not yet sufficiently depressed to attain maximum jelly strength. It seems clear that this effect is not the result of formation calcium bridges between carboxyl groups, since in that case an increase of gel strength should be expected.

Such an increase of gel strength has been reported by DOESBURG (125) and SÄVERBORN (416) after addition of small amounts of trivalent cations (aluminum, iron, cobalt), when using hydrochloric acid for acidification of the gels. This effect is not shown in the presence of organic acids which form complexes with these ions. When using hydrochloric acid these cations are causing a maximum gel strength at pH $\pm$ 2.7–3.3 (Fig. 17). The mechanism active in forming these kinds of jellies undoubtedly

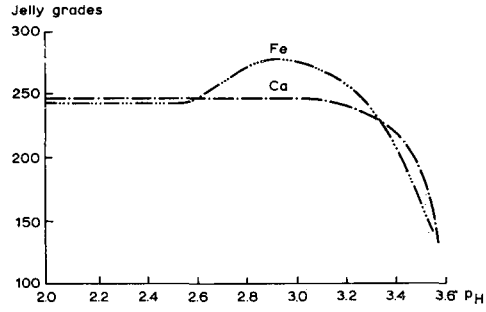


FIG. 17. Influence of iron (0.003 N in gels) upon relation between gel strength and pH. Degree of esterification of pectin 72% (Doesburg 125).

involves a new factor, which may be related to a strong depression of ζ-potential of pectin molecules by trivalent cations.

HARVEY (194) has studied the rheological behaviour of pectin gels by means of shear deformation, in relation to the common basic structure of these gels. The influence of bivalent metals on gel strength was markedly affected by their concentration relative to pectin, by pH value and by sugar concentration; the behaviour due to magnesium often differed from that due to calcium.

In close correspondence of the effect of cations on gel formation as shown in Fig. 15 and 17, HARVEY (193) stated that such an effect is found only when ionised carboxyl groups are present. Therefore the influence of cations is limited to relatively high pH-values, when the relatively high ζ-potential, caused by the presence of dissociated carboxyl groups, is partly reduced as a result of the influence of these ions.

A theory on the phenomena of the jellying of pectins, especially in regard to pH-conditions, has been developed by HINTON (214). Later this theory has been extended by HARVEY (193). HINTON has based his theory on numerous determinations of dissociation 'constants' of pectins under varying conditions. However, it has to be mentioned that DEUEL (106), when determining dissociation 'constants', has found other results; in contrast to the results of HINTON it was shown by DEUEL that the dissociation 'constants' are influenced by the degree of esterification.

Mostly pectin gels are produced by cooling after boiling of the pectin-sugar-acid mixture. However, heating of the mixture is not necessary for the formation of these gels.

Jellying phenomena without previous boiling have been studied by DEUEL and EGGENBERGER (113) by mixing sugar-pectin with sugar-acid solutions. The sugar concentrations in the finished jellies ranged from 42-49 %. Within the temperature range from 0–40 °C the rate of gel formation increased with increasing temperature; this is in contrast with the results at higher temperatures when cooling the jelly after boiling as has been reported by DOESBURG and GREVERS (143). According to the results of DEUEL and EGGENBERGER (113) gels formed at 25–30 °C were firmer than at lower temperatures. DOESBURG (125) has reported that gels prepared from boiling solutions develop an optimum gel strength when stored at 15–30 °C. The decrease of strength of gels manufactured or stored at low temperatures (0–15 °C) may be caused by a stronger kinking of pectin molecules.

In food manufacture, the *setting time* of a jelly batch is an important property. Setting time has been defined by DOESBURG and GREVERS (143) as the time between the moment that all ingredients necessary for forming the jelly are present in the heated solution in the correct proportion and the moment that jellification into a coherent mass occurs.

During cooling after boiling a temperature is reached at which jellification takes place rather suddenly. It has been shown by HINTON (215) that the temperature at which setting of the jelly occurs is influenced by the cooling rate; with a higher cooling rate the temperature of setting will be lower as is shown in Fig. 18.

According to the results of DOESBURG and GREVERS (143) this effect of 'undercooling' is influenced by the pH of the jellying mixture, the lower the pH value the less the 'undercooling' effect, as shown in Fig. 18. They measured the *setting temperatures* of purified apple pectins with different degree of esterification in jellies with varying pH, pectin content, ash contents and a sugar content of 65 %. The results are shown in Fig. 19.

When applying such a slow cooling rate (0.4 °C/min.) it may be expected that the

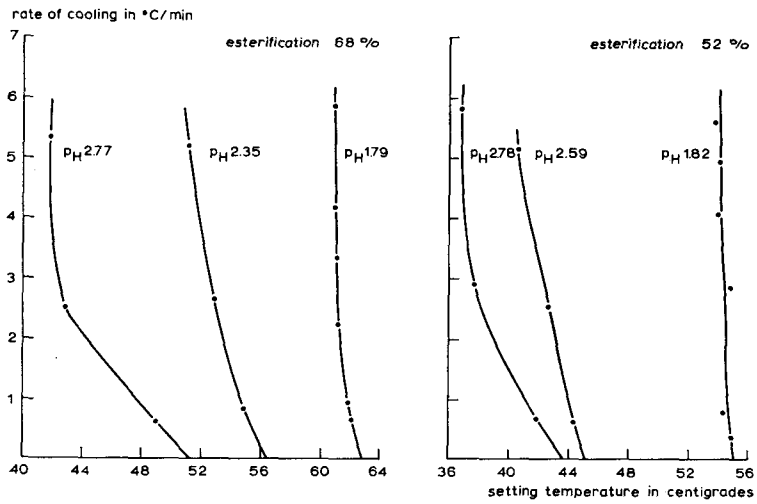


FIG. 18. Influence of the rate of cooling on setting temperatures of gels with different pH, made from two pectins with different degree of esterification. (Doesburg and Grevers, 143).

'undercooling' effect is very small; the setting temperatures have shown to be very reproducible for every set of conditions.

The results from Fig. 19 indicate that within a wide range of pH a linear relation exists between setting temperature and pH as well for the gels with and without addition of calcium salts or apple juice. The slope of the curves, indicating this relation, is nearly the same for all pectins. Without addition of calcium salts the setting temperature is lowered by decrease of degree of esterification. Pectins with a degree of esterification of about 80% may show jellification during boiling of jellies at pH 3 or lower.

The influence of the addition of calcium on setting temperatures is dependent also on the degree of esterification of pectins. It is important to note that the setting temperatures of jellies from apple pectins having the lowest degree of esterification (53%) is raised considerably by the addition of calcium and under these circumstances is approaching the setting temperatures of pectins with a degree of esterification of 75% (Fig. 19). From the results with apple pectins having degrees of esterification of 53% and 75% it may be concluded that a large increase of pectin concentration causes only a small rise of setting temperature.

According to the experience of PILNIK (377) and DOESBURG (140) citrus pectins don't show such a strong relationship between their degree of esterification and setting time. This somewhat different effect of degree of esterification of citrus pectins may be related to a slightly different distribution of esterified carboxyl groups over the pectin molecules. HERI (205a) reported that, in relation to the degree of esterification, apple pectins were found to be more homogeneous than citrus pectins.

HINTON (215) has found a lack of correlation between changes in setting temperature and jelly strength. As it can be seen from Fig. 19 the setting temperature is decreasing over the pH range from 2.0 to 2.6 where for all pectins a constant gel strength exists (Fig. 15). Nevertheless, it is clear that the sudden fall of setting temperatures at a higher pH than about 2.8 for jellies made from the pectin with 53% esterification

without $CaCl_2$ or only with the addition of apple juice, is connected with the decrease of jellying power under these circumstances as shown in Fig. 15.

HINTON (215) claimed that the setting temperature of a jelly is shown to be a determinate physical property. He indicated that the points at which the temperature curves in Fig. 18 cut the temperature axis (i.e. setting temperature at infinitely slow cooling) should coincide with the re-melting temperature of the gel. This effect has not been found by DOESBURG and GREVERS (143); the gels from Fig. 18 could not be remelted by heating to 100°C.

According to HINTON, in contrast to setting temperature, *the setting time* should be regarded as a secondary property dependent on temperature conditions. However, according to DOESBURG and GREVERS (143) it is doubtful if it is possible to indicate accurately for any set of conditions the highest temperatures at which jellification into a coherent mass can be completed. They stressed that for a definite set of conditions setting temperature depends on setting time. Really it is questionable if a sharp setting temperature as a determinate physical property may be found by extrapolation to infinitely slow cooling.

From results presented by DOESBURG and GREVERS (143) it seems reasonable to

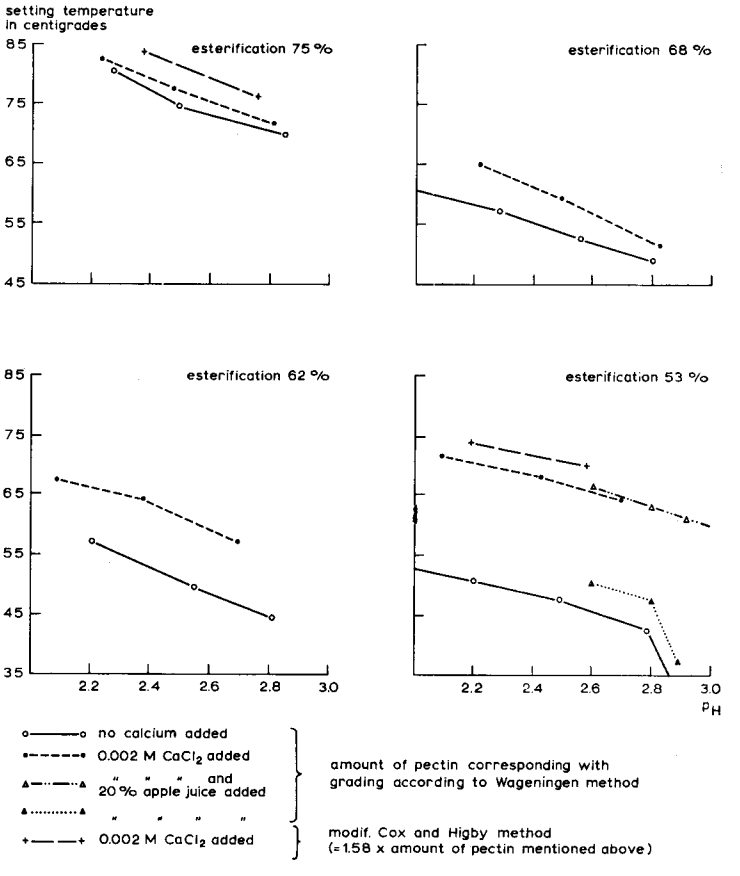


FIG. 19.
Relation between setting temperature and pH in gels from pectins with different degree of esterification during cooling at 0.4°C./min. (Doesburg and Grevers, 143).

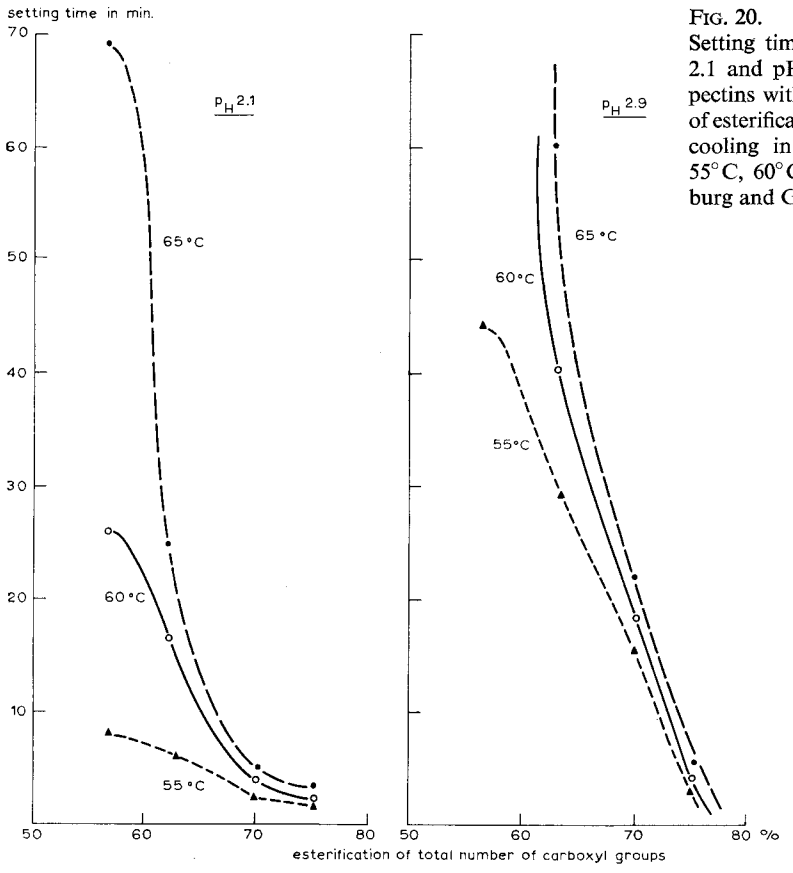


FIG. 20.
Setting times of gels at pH 2.1 and pH 2.9 from apple pectins with different degree of esterification, during rapid cooling in a waterbath to 55°C, 60°C or 65°C (Doesburg and Grevers, 143).

conclude that a jelly mixture shows a different velocity of the setting 'reaction' at different temperatures, infinitely slow at relatively high temperatures and increasing with decrease of temperature within the temperature range of $\pm$ 100°C–50°C. Within a certain set of conditions (sugar content, pH, ash constituents) the level of temperature range at which the setting time changes from infinitely long to a short period is strongly influenced by the degree of esterification of the pectin used (Fig. 20).

During cooling at a certain rate, jellification starts at a certain temperature and develops faster at lower temperatures. At a slow cooling rate the setting 'reaction' has the opportunity to proceed further at higher temperatures than with rapid cooling. In other words, with slow cooling the integration of the setting 'reaction' during the longer period at relatively high temperatures causes setting into a coherent mass at higher temperature than during rapid cooling. In this way it is possible to explain the 'undercooling' found by HINTON (215).

In Fig. 18 it is shown that these 'undercooling' effects increase with rise of the pH of the jelly mixture. From these facts it is concluded that at higher pH values there should be a relatively slow increase of velocity of jellification with decrease of temperature, whereas at low pH-values the change of setting velocity from infinitely slow to very rapid should occur within a rather narrow range of temperatures. The strong depression of ζ-potential by decrease of pH may be taken as an explanation for this different behaviour.

From the reported results it is impossible to deduce at which temperature the formation of a jelly is starting. Therefore it should be necessary to measure setting times over very long periods and in many cases it is to be expected that, under these conditions, partial decomposition of the pectin is occurring. Also it is not known if the same types of bondings are involved in the earlier and later steps of jellification.

Measurements of setting times have been made (143) by cooling the jellies made from apple pectins with different degree of esterification after rapid cooling to constant temperatures. In these experiments the test tubes with jelly mixture were placed immediately after filling into a vigorously stirred waterbath at constant temperature.

The time required for the mixture in the tubes to reach the temperature of the waterbath was noted. When the temperature of the waterbath was 65° C, 60° C or 55° C the times needed to cool the inner part of the test tubes to these temperatures showed to be respectively $4\frac{1}{2}$, $4\frac{3}{4}$ and 5 min. Results of these trials with jellies from pectins with different degrees of esterification at pH 2.1 $\pm$ 0.05 and pH 2.9 $\pm$ 0.05 are shown in Fig. 20.

It is clear that setting time is increased by a rise of pH of the jellies which has been shown also by DOESBURG (125), JOSEPH and BAIER (242) and KERTESZ (259). It is remarkable that for some sets of conditions rather long setting times have been noted (60 min.) which are easily reproducible with an accuracy of $\pm$ 1 min, which is reported also by PILNIK (377).

As has been stressed in the foregoing discussion there is no doubt that setting time at constant temperatures has to be regarded as a real property of a jellying mixture for a special set of conditions. Whereas the setting time is strongly increased with increase of pH, it has to be expected that setting time is very long in the neighbourhood of the maximum pH of jellying, even at low temperatures (room temperature). This may account for the fact that the stiffening of jellies with such a high pH may take several weeks as has been shown by LAMPITT and MONEY (276) and DOESBURG (125).

For practical purposes (to determine the characteristics of a pectin preparation) measurements of setting time as well as setting temperature may be very useful.

It has to be pointed out here that the relation between jelly strength and pH may be influenced by the setting time. When during cooking or cooling and filling the structure of the forming jelly is disturbed the strength of the finished jelly will be lowered as a result of *pre-setting*. Since setting time decreases by lowering of pH, this effect will be found mainly in jellies with relatively low pH-values. An example of such a changed relation between pH and jelly strength is given in Fig. 21, which has been taken from the results of BAKER and WOODMANSEE (28).

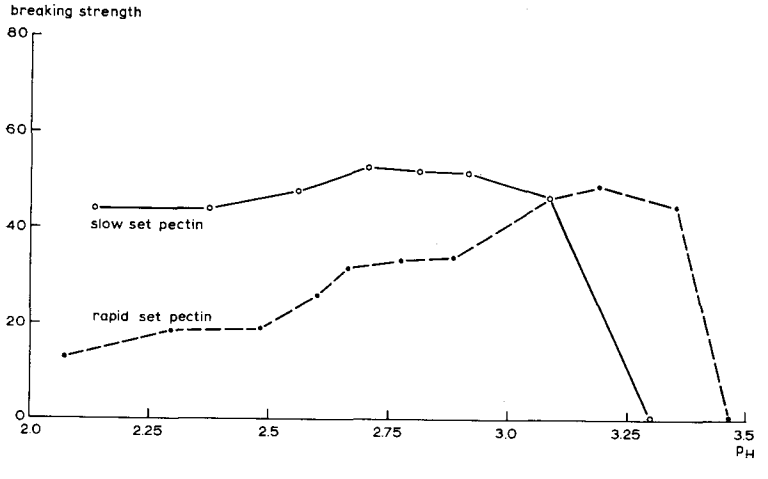


FIG. 21. Influence of pre-setting upon the relation between optimum jellying power and pH of a slow set and a rapid set pectin (Baker and Woodmansee, 28).

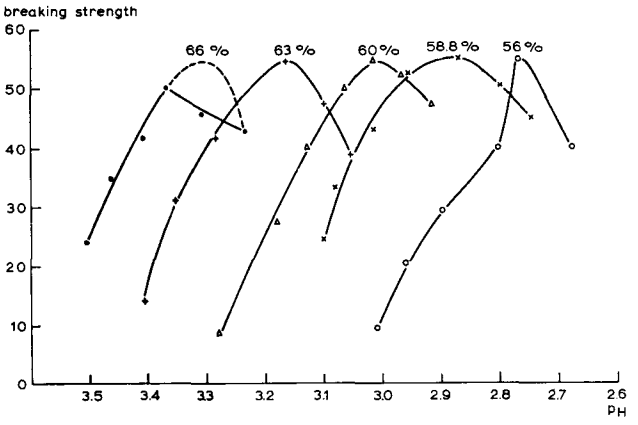


FIG. 22.
Different pH-values of optimum gel strength, influenced by different sugar concentrations (Stuewer, Beach and Olsen, 465).

This relation will be altered with variation of conditions of cooking, cooling and filling. Generally it may be stated that the longer the time between cooking and filling of jars or the more intensive the cooling, the effect of this pre-setting of jellies will be greater.

The effect of pre-setting of gels will depend also on increasing sugar contents, since the setting time is shortened by increase of soluble solids. It has been mentioned already that the maximum pH of jellification is increased by higher sugar contents. For these reasons within a given set of conditions (cooking time, cooling, filling) the *optimum pH for jellification*, as shown in Fig. 21, is also raised when the sugar content is higher. Results of experiments on this subject by STUEWER, BEACH and OLSEN (465) are presented in Fig. 22.

Since setting time or setting temperature is governed by the degree of esterification and for pectins with a relatively low degree of esterification also by the calcium content of the jelly, for the development of maximum jelly strength pectins have to be chosen which are suitable for the special set of conditions under which they are used. When taking into account the influence of natural calcium contents of foodstuffs the rather high setting temperature of pectins with relatively low degree of esterification has to be regarded. According to their degree of esterification high-methoxyl apple pectins as used in foods can be divided into various types as shown in Table 3 (139).

TABLE 3. *Relation between degree of esterification and setting time of apple pectins.*

Degree of esterification	Type of pectins
80 – 82 %	ultra rapid set
74 – 76 %, or 50 – 55 % (with calcium)	rapid set
68 – 72 %	medium rapid set
60 – 65 %	slow set

When aiming at even distribution of fruits in jams, cooked in open pans, it will be useful to apply rapid-set pectins and relatively small jelly batches; in this way fruits are rapidly enclosed by the forming jelly and kept in equal distribution during filling of jars. When manufacturing jams at lower temperatures in vacuum pans slow-set pectins are suitable for this purpose. Slow-set pectins and larger batches may be used also in the manufacture of jellies where the distribution of fruits has not to be taken into account.

It has been mentioned already that there is a rather close relation between degree of polymerization and jellying power of pectins, whereas jellying power may be influenced also by degree of esterification.

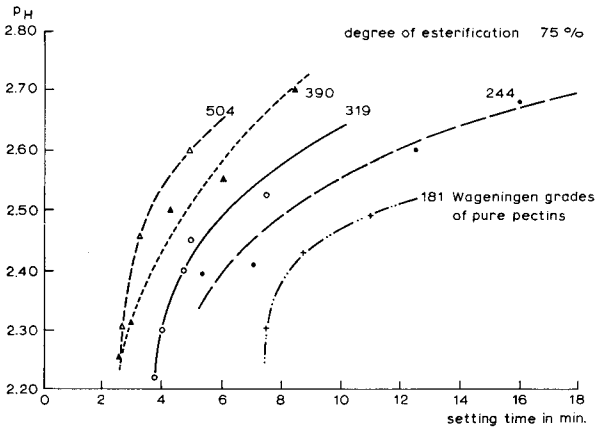


FIG. 23.
Relation between setting times and pH of jellies with different jelly grade (degree of polymerization) prepared by action of ascorbic acid and hydrogen peroxide (Doesburg and Grevers, 143).

It has to be pointed out here that the degree of polymerization has also an effect on setting time. DOESBURG and GREVERS (143) have studied extensively the influence of degree of polymerization by preparing pectins with varying degree of polymerization but the same degree of esterification. Three different methods of depolymerization have been used. When preparing gels with the same final strength from these partially depolymerized pectins they found that in all cases the setting time was enlarged by depolymerization. Some results of these experiments are shown in Fig. 23, where the setting times of depolymerization products, produced by treatment with ascorbic acid and hydrogen peroxide, are given. Jellies with the same final strength have been made from each pectin at several acidities. The setting time test tubes were cooled in air and the extent of cooling was measured by thermocouples. Setting times of 2, 6, 10 and 14 minutes at the setting time axis correspond respectively with 91.0, 75.0, 62.8 and 54.0°C. The differences of degree of polymerization of pectins are indicated by their grade values; the lower the jelly grade value the lower the degree of polymerization.

Syneresis in commercial jams and jellies is regarded as a defect, since it leads to separation of syrup when the jelly is cut or broken in use. SWAJKAJZER (470) studied the effect of total solid concentration and pH. When using commercial slow-set apple pectin, syneresis in gels at pH 3.2 showed to be not affected by total solids concentration. At pH 2.6 the syneresis increased when total soluble solid concentration was reduced. When the mixture at the end of the boil was adjusted to pH 3.2 no influence on syneresis could be found of boiling with sugar; the syneresis increased in mixtures similarly adjusted to pH 2.6.

Low-methoxyl pectins

Low-methoxyl pectins can be used for the formation of gels with a low soluble solids content as well as for gels with a high soluble solids content. For the production of gels from low-ester pectins the presence of calcium is necessary.

Contrary to the systems of high-methoxyl pectins plus sugars, these low-methoxyl gels are mostly described as principal valence gels (190, 305, 363). According to OWENS, MCCREADY and MACLAY

(363) the presence of calcium bonds in the low-solids gels is hardly questionable; the fact that low-solids gels are brittle should coincide with expectations for electrocovalent links (427, 451). From these and other facts OWENS ET AL (363) are concluding that low-methoxyl gels are formed through calcium bridges between carboxyl groups supplemented by hydrogen bonds. HARVEY (194) showed that the influence of bivalent metals on strength of pectin gels was markedly affected by their concentration relative to pectin, by pH value and sugar concentration.

For the same reasons as mentioned in the discussion on protopectin structure (see p. 17) it seems unlikely that calcium bridges are occurring in gels from low-methoxyl pectins, which has been postulated also in relation to jelly formation by high-methoxyl pectins. In relation to this it has to be remembered that precipitation of pectinic acids with moderate degree of esterification requires 4–5 times the quantity of calcium calculated from the free carboxyl groups, whereas according to OWENS ET AL (363) the number of milligrams of calcium per gram of low-methoxyl pectin needed in water gels is dependent on the manner of preparation of these pectins (deesterified in acid or alkaline milieu or enzyme-demethylated). HAMM (190) has made the suggestion that there is indeed no normal salt-like bond, but calcium could possibly be bound as a chelate; in this case the binding of calcium and its cross-linking effect would depend on the steric position of the pectin molecules. According to HARVEY (194) the presence of calcium promotes the formation of pectin gels by forming either covalent bonds or strong ion-associations with carboxyl groups of neighbouring pectin chains ('salt bridges').

As mentioned before the amount of calcium per gram of low-methoxyl pectin needed in water gels is influenced by the manner of preparation of these pectins. According to JOSEPH, KIESER and BRYANT (243) the number of milligrams calcium ions is usually 4–10 per gram of enzyme-demethylated pectins, 15–30 for pectins made by the ammonia-ethanol process (see p. 51) and from 30–60 for the ones made by saponification in acid (see p. 45). The amount of calcium required to obtain low-solids gels at methoxyl contents near 5 percent is considerably higher than when methoxyl contents are near 3 percent.

Since calcium is responsible for jellification it is evident that one would expect to obtain the best solution of the pectin when no calcium is present. As it has been pointed out by JOSEPH, KIESER and BRYANT (243) the ideal way to get a gel would be to dissolve the pectin, then while the sol is hot, a solution of some calcium salt has to be added. After cooling such a sol to room temperature a gel of good texture will result. This procedure is not always possible from a practical point of view since it may be necessary sometimes to have the calcium salt present with the pectin at the start of the operation. For that reason the use of slowly or slightly soluble calcium salts are desirable. Monocalcium phosphate, $Ca(H_2PO_4)_2$. H_2O and tricalcium phosphate are good salts for this purpose, because with these salts not enough calcium is immediately available to interfere with the solution of the pectin.

HILLS ET AL (212) explained the differences in jellying characteristics of enzyme- and acid- deesterified pectins on the basis of fundamental differences in composition and structure of both products. Acid catalysis causes the simultaneous removal of methyl ester groups and non-galacturonide materials. Enzyme- deesterification does not affect the non-galacturonide materials and causes a non-random attack of esterified groups as will be discussed later (Chapter 4).

Too high a calcium content of low solids jellies may cause syneresis of jellies as shown by BAKER and GOODWIN (23).

Jellies prepared from low-methoxyl pectins usually have a 0.5–1 % pectin content. As reported by MCCREADY ET AL (320) these pectins can be used in gelled fruit cock-

tails as well as in milk puddings and other products, which present considerable variation in pH, calcium content and type of solids.

According to HILLS ET AL (209) the best results are obtained with pectins with a methoxyl content ranging from 3.5–6.0 %. From the evidence presented by OWENS ET AL (363), alkali-deesterified pectins containing less methoxyl than about 2.5 precent may be so sensitive to calcium ions that they cannot be used in fruit juices or even in dessert powders where the water to be added may contain high quantities of calcium salts. At methoxyl contents above 3 percent, little difficulty has been experienced with calcium sensitivity or with syneresis unless the total solids are reduced to near zero.

The application of low-methoxyl pectins in various products, ranging in pH from $\pm$ 3 to $\pm$ 7, is possible from their jellying properties. The relation between firmness of jellies and pH is shown in Fig. 24 (140). The compression modulus Ec has been calculated from the sag shown by cylindrical gels (125) standing upon their bases after being taken out of their containers. The finished gels contained 30% sugar, 1% pectin, 0.1% sodium citrate and 0.07% $CaCl_2$; the pH was varied by addition of different amounts of citric acid. A similar relation between shear modulus of gels and their pH-values has been found by OWENS ET AL (363) in watergels with varying ratios of calcium to pectin. Over the whole pH-range the firmness increased with the calcium content till an optimum calcium/pectin ratio was reached. From Fig. 24 it is clear that, at relatively high pH-values, more pectin is needed to produce a firm jelly than at lower pH-values.

Finally it should be mentioned that the firmness of gels from low-methoxyl pectins is markedly influenced by temperature; the melting points of low solid and high solid jellies range from about 35 °C to 60 °C. It has been pointed out by OWENS ET AL (363) that products to be shipped to warm regions or to be used at relatively high temperatures will need to be prepared with more pectin or with a higher calcium to pectin ratio than called for under cooler conditions. Low-solids gels from low-methoxyl pectins are considerably more brittle than sugar-acid gels from high-methoxyl pectins.

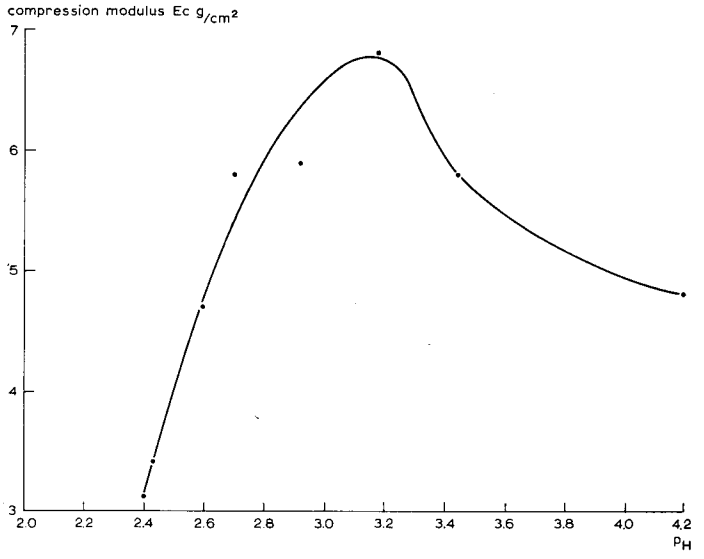


FIG. 24.
Relation between pH and gel strength (compression modulus of elasticity) of a pectin with a degree of esterification of 32% (Doesburg, 140).

The stability of pectic substances in solutions; influence of pH and temperature; deesterification

According to KERTESZ (259) solid pectinic acids with less than 10% moisture content change little during long periods of storage. Solubilized pectic substances, however, may show gradual irreversible changes, often designated as 'ageing', or degradation.

These alterations, which affect viscosity and jellying power of the solutions, are caused by: 1. decrease of degree of polymerization of polygalacturonic chains. 2. decrease of degree of esterification of pectinic acids. 3. changes in the non-uronide materials attached to the polygalacturonic chains.

Mostly these changes are dependent on pH and temperature of the solutions; however, in some cases they are known to be caused by reactions with other components present in the solution (see p. 51). First, reactions which are mainly dependent on the pH will be described. As pointed out by KERTESZ (259), during heating, solubilized pectinic acids display their greatest stability when the pH is 3–4. It has been shown recently that the decrease of degree of polymerization at more elevated pH-values is caused by a reaction mechanism other than the hydrolysis of polygalacturonic chains in acid milieu. Moreover, the influence of various temperature and pH-combinations will vary since the rate of different reactions is not influenced to the same extent.

Acid (pH<4) solutions

DOESBURG (140) studied the changes of degree of esterification, jellying power and amount of apple and citrus pectins in concentrated solutions at various pH-values (ranging from 1.8–3.0) during storage at 15°C and 25°C. The results with apple pectin are shown in Fig. 25; the polygalacturonic content, estimated by titrimetrical method (131) has not been plotted, since under these conditions it was found to be

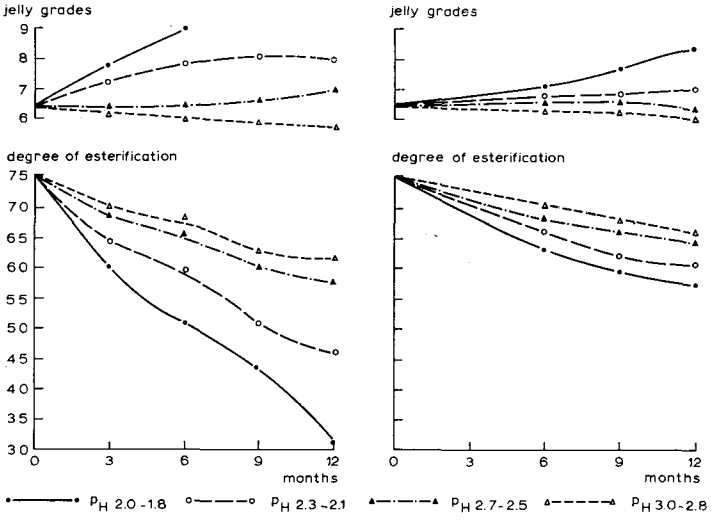


FIG. 25.
Changes of degree of esterification and jellying power of pectins in solutions at pH 1.8–2.0 to 2.8–3.0, stored at 15°C (right) and 25°C (left) (Doesburg, 140).

constant. Experiments with citrus pectins yielded similar results. As expected, the decrease of esterification during storage is promoted by decrease of pH and elevation of temperature. During storage at 25°C the jellying power of the pectin concentrates is slightly diminished at pH 3.0–2.8, whereas it is increased by decrease of pH. At 15°C, the jellying power is decreased somewhat when the pH of solution is 3.0–2.8 or 2.7–2.5 and increased at pH 2.3–2.1 or 2.0–1.8. When comparing the relation between degree of esterification and the jellying power of pectins produced after storage under different conditions, it can be concluded that pectins with the same decrease of degree of esterification, do not show the same jellying power. From these results, it is clear that jellying power not only depends on degree of esterification, but that at least one other factor is involved.

It has been mentioned already (see p. 33) that jellying can be enhanced under conditions which promote partial deesterification under suitable conditions. This effect has been shown also in Fig. 15 as a result of partial saponification at pH 1.0 and 60°C or by treatment in alkaline milieu (p. 50).

To produce slow-set pectins or rapid-set pectins with a relatively low degree of esterification and low-methoxyl pectins it is necessary to effect a partial saponification without severe depolymerization of polygalacturonic chains, which will cause a decrease of jellying power. When applying suitable conditions, however, the jellying power of the same batch of pectin may be increased as shown before (Fig. 15 and Fig. 25).

It is not known exactly if this promotion of jellying power is a result only of the formation of a greater number of free carboxyl groups or whether the removal of non-uronide materials has to be taken into account also. It has been mentioned earlier that the elimination of acetyl groups is essential for the production of high grade beet pectins. To a lesser extent, however, the same effect may be caused by the removal of non-uronide materials from apple or citrus pectins under such conditions as has been discussed earlier.

When the ratio of the velocity of deesterification and of hydrolysis of polygalacturonan chains in acid milieu is decreased, the jellying power of deesterification products will decrease also. Such conditions may occur when more elevated temperatures are applied to speed up the saponifications. This subject has been studied extensively by BAKER and GOODWIN (22) and WOODMANSEE and BAKER (521) in relation to production of slow-set high-methoxyl pectins and low-methoxyl pectins. Several modifications are mentioned also by MAASS (294). These saponifications take about 10–15 hours for the production of slow-set pectins and 40–50 hours for low-methoxyl pectins. It has been shown to be useful to combine such treatments with the extraction of pectins (294).

Such a procedure for the extraction of pectins, from apple pomace, ranging in degree of esterification but of high molecular weight, has been developed by OLSEN and STUEWER (358). The dehydrated apple pomace or other suitable raw material is mixed 1:2 (v/v) with hydrochloric acid of such concentration that the resulting mixture is at about pH 1.0 and still essentially is a solid. After being held 24–48 hours at 40–41°C (so called pickling), the mixture is diluted with 15–20 parts of water, adjusted to about pH 3.0 with sodium carbonate or sodium hydroxide, and held 1–2 hours at 60°C. Under these conditions the acid extract can readily be separated from the residue by pressing.

The kinetics of deesterification of pectinic acids in acid milieu were studied by MERRIL and WEEKS (336). The deesterification is a first order reaction with respect

to pectin concentration. This is shown by the fact that the fraction of reaction completed in a given time is independent of the pectin concentration.

The disadvantageous change of the ratio of velocities of deesterification and hydrolysis of polygalacturonic chains when applying more elevated temperatures is shown in Fig. 26 (140). The amounts of polygalacturonides have not been plotted, since they remained constant during this treatment. During heating at 85°C and pH 1, the deesterification is proceeding rather rapidly but causes also a severe loss of jellying power, which is not counterbalanced by the influence of decrease of esterification.

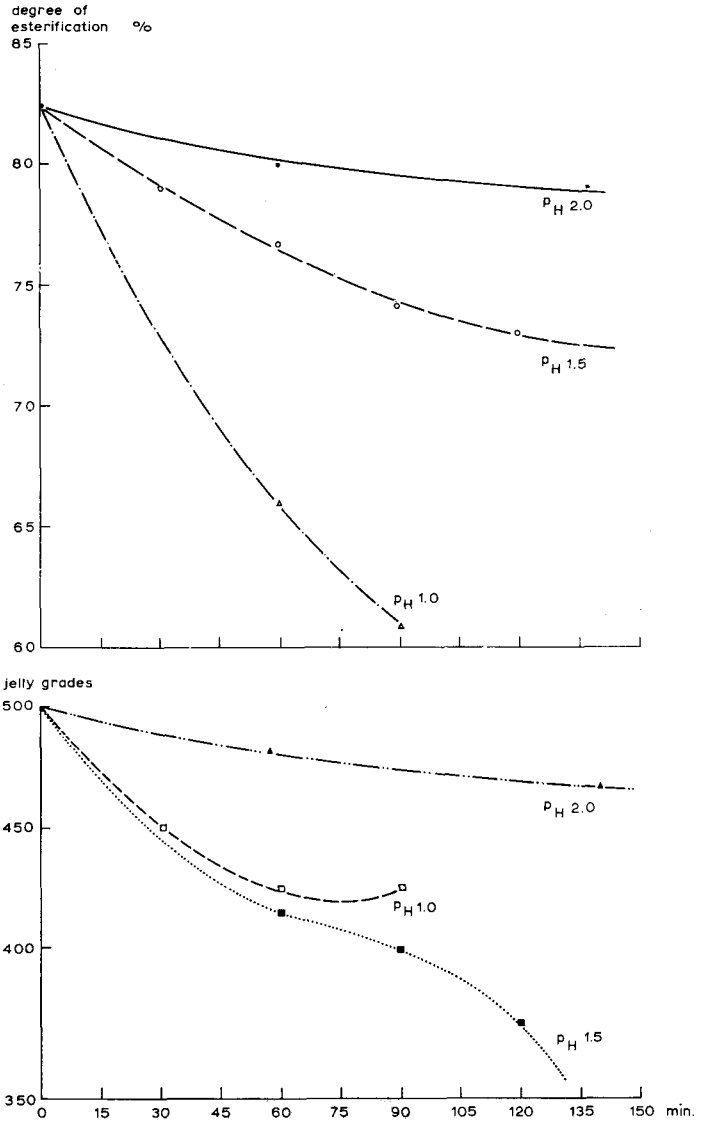


FIG. 26.
Influence of heating at 85°C on degree of esterification and jellying power of apple pectin in solutions at pH 2.0, 1.5 and 1.0 (Doesburg, 140).

At pH 1.5 the saponification takes place slowly and, when regarded in relation to the degree of esterification, the loss of jellying power is even more pronounced. At pH 2 the saponification as well as the loss of jellying power are strongly limited.

The breakdown of pectinic acids in solutions with pH-values ranging from 2 to 4 was studied by MERRIL and WEEKS (335); after prolonged heating they found a strong drop of viscosity.

Low-acid (pH 4–7) solutions

From these and other results, it may be concluded that pectins are rather stable during heating at pH 3–4 as already has been pointed out by KERTESZ (259).

However, when heating solutions of high-methoxyl pectins at more elevated pH-values a severe loss of jellying power as well as of the amount of polygalacturonides will occur; further a limited decrease of degree of esterification takes place as shown by DOESBURG and GREVERS (143). These effects are shown in Fig. 27 where the analytical data are plotted for pectins, which have been prepared by boiling aliquots of a 1.15% pectin solution (degree of esterification 75%) for 15 min. at pH-values ranging from 3.0 to 7.5. The solution was buffered by 0.37% lactic acid, citric and phosphoric acid, and before heating, the pH of each aliquot was adjusted to the different pH-values mentioned above by using 20% NH_4OH. Nevertheless, the pH changed in some cases during boiling to a lower pH value, probably caused by a partial decrease of esterification of the pectin at the higher pH-values. This is in contrast to a remark by KERTESZ (259) who has stated that it has been generally observed by many investigators that the acidity of pectinic acid solutions is increasing during heating, but that this increase of acidity does not appear to be a result of deesterification. From Fig. 27 it is clear that at higher pH-values decomposition of the pectin occurred as indicated by diminishing jelly grade at a pH higher than about 4, and a measurable decrease of pectinic acid content at pH higher than about 5.5. During prolonged heating, the last effect can be met with already at lower pH-values.

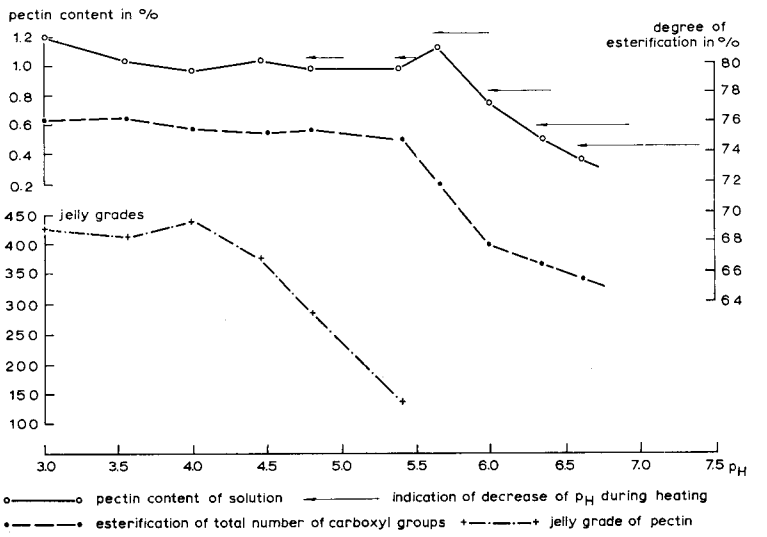


FIG. 27. Influence of boiling during 15 minutes at different pH-values on the jelly grade, degree of esterification and amount of pectin (Doesburg and Grevers, 143).

ALBERSHEIM (2) measured the influence of heating upon the viscosity of pectinic acid solutions with a pH ranging from 3–8 and showed that pectinic acids with a low degree of esterification are much more stable than pectinic acids with a high degree of esterification. For this reason, ALBERSHEIM concluded that the polyuronide breakdown seems to require the carboxyl groups to be esterified.

The mechanism of splitting of pectinic acid chain molecules in neutral or weakly acid solutions has been elucidated by ALBERSHEIM, NEUKOM and DEUEL (4), who showed that this degradation is also dependent on the presence of esterified groups. The rapid decrease in viscosity and increase in reducing end groups indicate a splitting of glycosidic bonds within the pectinic acid chain molecules. The reaction has a relatively high temperature coefficient, the Q_{10} between 50°and 95°C being approximately 3.5.

ALBERSHEIM, NEUKOM and DEUEL (14) have stated that the breakdown products formed by heating pectinic acids in neutral environment display an absorption maximum at 235 mμ, react with thiobarbituric acid to give a product which absorbs at 547 mμ (347) and, on treatment with ozone, give rise to the formation of oxalic acid. These observations strongly point to the formation of an unsaturated compound by means of a trans-elimination reaction which results in the removal of the hydrogen atom at C–5 and of the glycosidic residue at C–4, as shown in Fig. 28 (4).

The non-esterified carboxyl group at C–6 is not sufficient to activate the hydrogen at C–5, therefore the splitting of glycosidic bonds, as shown below in alkaline milieu, does not occur in sodium pectate.

Since the real influence of these reactions was recognized recently, it was not taken into account in earlier experiments and methods. Determination of pectic substances in plant materials should not be performed after extraction at high temperatures and relatively high pH-values (154, 364). Consequently no value can be attached to determination of the jellying power or molecular weight of pectin substances extracted in a similar manner (154, 416).

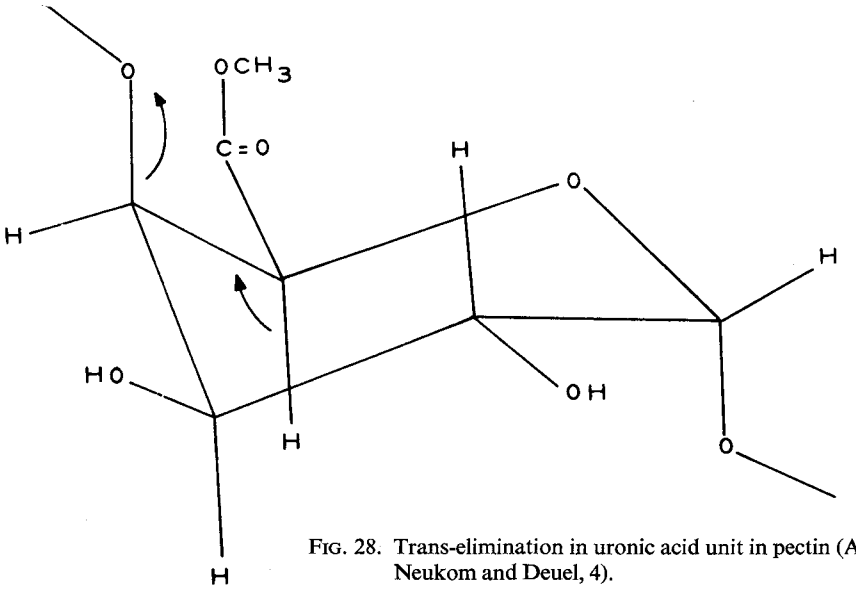


FIG. 28. Trans-elimination in uronic acid unit in pectin (Albersheim, Neukom and Deuel, 4).

According to ALBERSHEIM (2), the instability of pectinic acids at natural pH-levels suggests that, over extended periods of time, a pectinic acid may lose part of its ability to strengthen the cell wall. As proved by DOESBURG (131), however, during six weeks at room temperature, high-methoxyl pectins in solutions at pH 6 show no loss of jellying power and no changes in degree of esterification (Table 2). Therefore it is to be expected that the rate of degradation is strongly diminished at lower temperatures.

Alkaline solutions

Similar reactions take place in alkaline solutions of pectinic acids, even at low temperatures. VOLLMERT (499) has already shown that the decrease of viscosity observed during alkaline saponification of pectinic acid at 20 °C is due to the splitting of a few glycosidic linkages of the original polygalacturonide chains. The alkaline degradation of pectinic acids has been studied by NEUKOM and DEUEL (348, 349). They stated that the alkaline breakdown of pectinic acids increases more rapidly with increasing temperature than the concurrent saponification of the methyl ester groups.

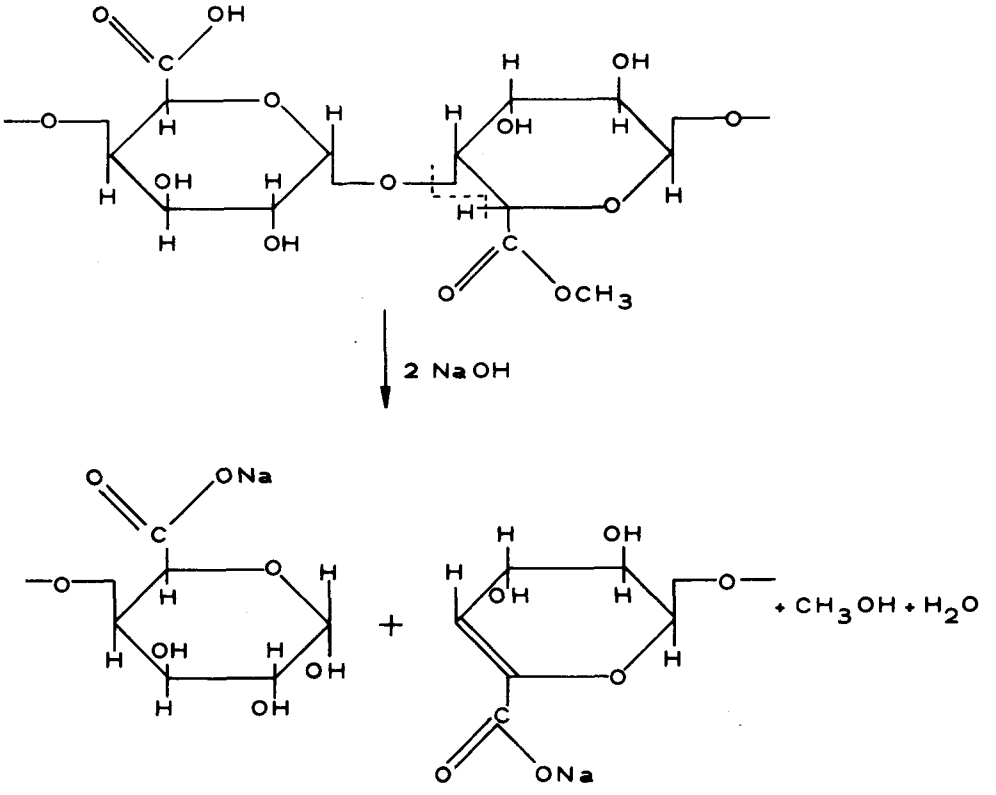


FIG. 29. Alkaline degradation of pectin. The glycosidic linkage in the β-position to the ester carbonyl group of pectin is cleaved following the removal of the activated hydrogen at C(5) and the formation of a double bond between C(4) and C(5) (Neukom and Deuel, 348).

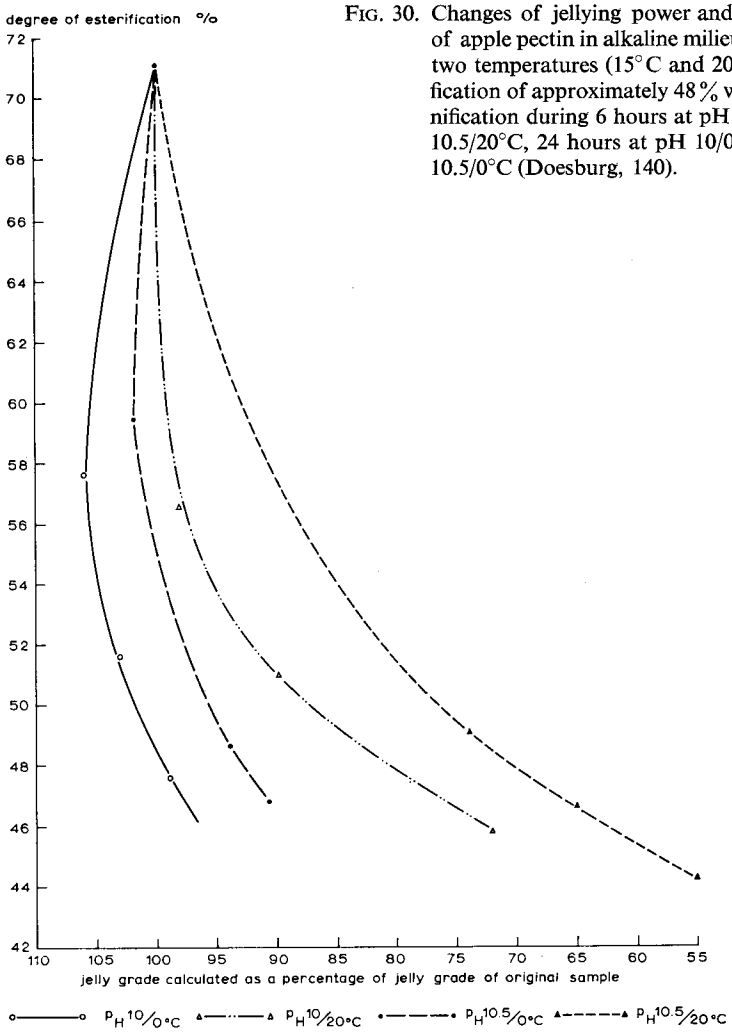


FIG. 30. Changes of jellying power and degree of esterification of apple pectin in alkaline milieu (pH 10 or pH 10.5) at two temperatures (15°C and 20°C). A degree of esterification of approximately 48% was produced after saponification during 6 hours at pH 10/20°C, 4 hours at pH 10.5/20°C, 24 hours at pH 10/0°C and 18 hours at pH 10.5/0°C (Doesburg, 140).

The same results have been recorded by SLAVIČKOVÁ (439) and BOCK and LANGE (55).

In alkaline environment the degradation is dependent also upon the degree of esterification of pectin; completely deesterified pectate is comparatively stable to alkali. The depolymerization reaction is shown in Fig. 29.

Saponification in alkaline milieu may be used for the manufacture of low-methoxyl pectins. It has the advantage that the deesterification may proceed rapidly. As has been described for the production of slow-set and low-methoxyl pectins by addition of acids, it is necessary to take into account that the ratio of the velocities of deesterification and depolymerization is also extremely important when applying a treatment in alkaline milieu.

When applying suitable conditions, partial saponification in alkaline environment

may cause also some increase of jellying power of the same original batch of pectin, as has been shown by DOESBURG (140). At more elevated pH or temperature, this resulting small increase of jellying power is readily surpassed by a loss of jellying power, which indicates that, under these conditions, the alkaline breakdown of pectins proceeds more rapidly than the rate of saponification (Fig. 30). This is in contrast with the results of BOCK and LANGE (55), who are stating that there is no marked influence of the pH-value (ranging from 9.0 to 10.3) on the jellying power of the prepared low-methoxyl pectins.

A description of the production of low-methoxyl pectins by deesterification in alkaline milieu has been given by MCCREADY, OWENS and MACLAY (320). In order to obtain useful preparations, a temperature of 5°C or below must be used at pH-values of 11 to 12. Further information on alkaline saponification has been collected by MAASS (294).

According to LINEWEAVER (283), the rate of saponification is influenced by the presence of various cations; the velocity of deesterification is increased by same amounts of cations in the following order: NH_4^+ and K^+, Mg^{2+}, Ca^{2+} (320).

DOESBURG (140) has studied the influence of additions of sodium salts during saponification with ammonia at pH 10. The rate of deesterification was promoted with increasing additions of salt, but the relation between alkaline breakdown and deesterification was not influenced by these additions.

JOSEPH, KIESER, and BRYANT (243) have described the effect of deesterification on a dry pectin powder suspended in ethanol containing ammonia or by exposure of the dry pectin powder in a desiccator to the vapours from ammonium hydroxide. Under these conditions, deesterification is accompanied by the formation of amides. The nitrogen is retained by the pectinic acids after purification (which would produce free carboxyl groups when deesterification had been performed with sodium or potassium hydroxide). According to KERTESZ (259) the resulting compound has methyl ester, carboxyl and amide groups and cannot be truly called a low-ester pectin.

Oxidative degradation of pectic substances

In the foregoing, the behaviour of pectic substances in water solutions was discussed in relation to the pH of these solutions. However, in the presence of oxidizing agents such as hydrogen peroxide, dichromate, permanganate, chlorine, bromine and periodic acid, complex reactions occur which may be designated as 'oxidative degradation'; according to PALLMANN and DEUEL (367), iodine and chlorine showed no such effects when tested in 0.01 M concentrations.

Special attention has to be paid to the action of ascorbic acid since it is naturally present in various products. The influence of ascorbic acid has been studied extensively by DEUEL (107). The oxidative degradation of pectic substances by ascorbic acid is only found in the presence of a suitable hydrogen acceptor. The reaction is very slow at pH 2 or below but is increased at higher pH-values.

According to DEUEL (107) the rate of the reaction is increased by larger proportions of ascorbic acid when the amount of oxygen dissolved in the solution is enough. Thus, shaking of the solution with air or oxygen is accelerating the reaction, whereas the degradation is prevented by an addition of hydrogen sulphide, sulphurous acid or sulphites which are stopping the oxidation of ascorbic acid. According to KERTESZ (259) the reaction is prevented also by the addition of an excess of iodine, presumably by the immediate complete oxidation of the ascorbic acid.

The oxidative degradation of pectic substances is promoted by the presence of substances which are catalysing the oxidation of ascorbic acid, e.g. copper and methylene blue, whereas methylene blue without ascorbic acid has no effect. In relation to the oxidative degradation of pectic substances the formation of hydrogen peroxide during decomposition of ascorbic acid may be important.

HERMANN and GROSSMANN (207) have pointed out that the copper-catalyzed oxidation of ascorbic acid is inhibited by the addition of pectins. The effect can be explained from the copper-binding properties of pectins, by which the amount of available copper is decreased.

According to DEUEL (107) the degradation of pectic substances influenced by ascorbic acid shows the same effect as the hydrolytic decomposition by pectolytic enzymes or the oxidation by periodic acid, but the reaction mechanism is not the same. A slight reaction causes a severe drop of the viscosity or jellying power of pectin solutions, whereas the degree of esterification and the amount of pectic substances are not affected as shown by DOESBURG (131). The results of the action of a pectolytic enzym and of ascorbic acid and hydrogen peroxide are shown in Fig. 31.

In the experiment with the enzyme, 2 g of a commercial enzyme preparation (Filtragol) were extracted for ½ hour at 35° C with 500 ml of water and filtered. 450 ml of filtrate were added to 450 ml of a pectin solution containing 1.47 % pure apple pectin (degree of esterification 60 %) with the pH adjusted to 3 by addition of a few drops of 25 % HCl. At different times after the addition of the enzyme extract, degree of esterification and jellying power of the pectin were determined.

In the experiments with H_2O_2 and ascorbic acid, a similar pectin solution, adjusted to pH 6 with a few drops of 20 % aqueous NH_3, was used. To 100 ml portions of the solution 100 ml water containing increasing amounts of ascorbic acid and H_2O_2 were added; after 2 hours the reaction was stopped by adding 2 ml of 6 % SO_2 solution and the pectin content, degree of esterification and jellying power were determined.

In both experiments no decrease of pectin amount has been recorded as would be found after prolonged standing or higher enzyme or H_2O_2 and ascorbic acid concentrations. However, such a rather weak action of pectolytic enzymes or other agents can be easily demonstrated by the use of jellying power or viscosity measurements as shown in Fig. 31.

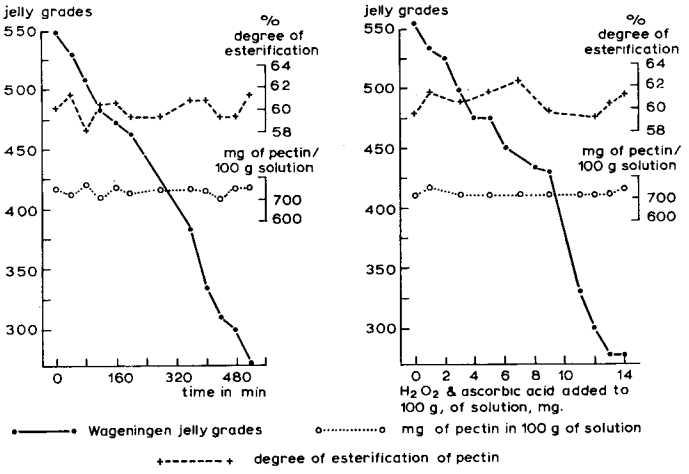


FIG. 31.
Degrading of pectin by pectolytic enzymes (left) or increasing amounts of H_2O_2 and ascorbic acid during different times (Doesburg, 131).

Chapter 3

Detection, determination and characterization of pectic substances

Detection of pectic substances in plant tissues

The detection of pectic substances *in situ* has been performed mostly by use of staining agents. In the earlier years more use of staining techniques has been made than in the recent investigations. KERTESZ (259) stated in 1951, that there is a great need for systematic investigation of the histological methodology of the detection of pectic substances but apparently little work has been done for a number of years.

A number of staining reagents has been introduced by MANGIN (296), including safranin, night blue, methylene blue and naphtalene blue, and some years later (297) ruthenium red (dilute ammoniacal solution of ruthenium oxychloride).

Little is known about the action of these staining reagents in the cell walls and there is real doubt that they are highly specific. Some work on the action of ruthenium red, which has been most extensively used by several investigators, has already been done by TETLEY (475). It was shown that pectic substances extracted from plants are stained only by ruthenium red when treated with alkali which causes saponification of methyl ester groups. From these observations it may be supposed that the staining reaction is limited to pectates or low-ester pectinic acids. It has been pointed out by NORTHCOTE (352) that ruthenium red will stain compounds similar to pectin which contain carboxyl groups and this includes some of the hemicelluloses such as xylans. According to CZAJA as cited by ANDERSON (7) the staining of the intercellular layer is not caused by pectic substances but only results from the presence of salts of calcium and phosphoric acid! CZAJA showed that cell wall materials can be stained with ruthenium red after having been ashed but without dislocation of the ash. Similar observations have been made by VAN GELDERMALSEN-DE JONG (175).

Rather recently MCCREADY and REEVE, (327, 395) introduced a new method for staining of pectic substances which may be applied equally well for determining esterified pectins in solid mixtures or as a histochemical test. The method depends on the reaction of the ester groups in pectic substances with aqueous alkaline hydroxylamine at room temperature which produces hydroxamic acids. Under these conditions esters of pectic substances react at a more rapid rate than deesterification takes place. The colour is formed, after addition of ferric ion, by formation of an insoluble coloured ferric hydroxamic acid complex. According to the authors the test is specific and requires only a few minutes. From the results of further work by GEE, REEVE and MCCREADY (174) it was postulated that these series of reactions can be applied as a direct method for following the esterification of pectic substances in fruits at different stages of their development. It is striking that the results, pertaining to peaches, apples and pears show the presence of highly esterified pectic substances *in situ* (degrees of esterification ranging from 70–100%) in these fruits.

However pectic substances extracted from fruits are known to have from 60–80% of their carboxyl groups esterified with methanol. Such of a high degree of esterification as found by GEE ET AL (174) has been reported only by SCHLUBACH and HOFFMANN-WALBECK (419) for pectins extracted from apples. Further evaluation of this staining method will be needed to conclude if the discrepancy between the results of estimation of degree of esterification of extracted pectic substances or *in situ* is a real difference which might be caused by extraction and purification of pectic substances.

Extraction

According to KERTESZ (259) the term 'total pectic substances 'covers all polygalacturonides whether originally soluble in water or not, which when brought into solution, will satisfy the criteria in the definitions in Chapter I. Thus 'total pectic substances' will include protopectin, pectinic and pectic acids, as well as pectates and pectinates.

When these 'total pectic substances' are known to have a high degree of esterification the extraction procedure is quite simple, by extracting the finely ground tissue with 0.05N hydrochloric acid at 80°C using 30 minutes for every extraction. The completeness of extraction can be easily ascertained by mixing a small amount of the cooled extract with ethanol in a 1:2 ratio; in the latest extract no flocculation should occur.

According to KERTESZ (259) the above mentioned extraction procedure is to be preferred to extraction with ammonium oxalate (0,5%) or other extractants.

However, when dealing with 'total pectic substances', which are expected to contain a more than a negligible amount of pectic acid, or low-methoxyl pectinic acids, extraction cannot be completed in acid media, since these substances are insoluble under these conditions. For solubilization of these low-methoxyl pectic substances, extraction at higher pH-values (± 6) is necessary in the presence of sequestering agents, to prevent flocculation by polyvalent cations.

Moreover, for reasons discussed in Chapter 2 (see p. 48), at pH-values higher than about 4, extractions have to be performed at a temperature lower than 60°C, since at higher temperatures pectic substances are liable to degradation, especially when they are showing a relatively high degree of esterification. When this effect is not taken into account during extraction, significant decrease of the amount of pectic substances may result by contrast with the action of heating in acid milieu.

In the author's laboratory it has been shown that the extraction of low-methoxyl pectic substances at pH 6 and 60°C and in the presence of sequestering agents can be promoted by a preceding heat treatment in acid milieu (as described before) to disrupt anchorage of these low-methoxyl substances in the cell wall. The beneficial effect of this pre-treatment was found when preparing low-methoxyl pectic substances from pea seed coats (140). It is clear that the influence of such a treatment is dependent on protopectin structure. When the insolubility of esterified pectic substances in the tissue is due mainly to the presence of polyvalent cations then extraction with sequestering agents alone will be sufficient, whereas a hot-acid pre-treatment will be neces-

sary whenever bonding and entanglement with other cell wall constituents is also an important cause of insolubility.

When the low esterified pectic substances in the tissue are insoluble solely as a result of flocculation by polyvalent cations, it is possible to extract them with sequestering agents before extraction of high-methoxyl pectic substances by a hot-acid milieu.

Purification

The extracts are filtered clear of suspended particles and the filtrate is used for the purification and determination of pectic substances.

Purification and concentration can be performed by precipitation in ethanol or acetone, thus permitting the removal of substances which may interfere in subsequent determination of pectic substances. It has already been pointed out in Chapter 1 that the amount of non-uronide matter is influenced by the manner of precipitation; the same holds for the amount of pectic substances which are precipitated, especially when there is a marked heterogeneity of molecular weights. As we have seen in Chapter 1 the lower limit of molecular weight of pectic substances is not yet sharply defined. In the definitions this difficulty has been evaded by stating that the polygalacturonides should have colloidal nature, but as a matter of fact no sharp separation between pectic substances and lower polygalacturonides can be recognized. In the case of considerable heterogeneity, fractionation of pectic substances may occur as a result of precipitation by varying the strength of ethanol or acetone as shown by PEYNAUD (375).

The lowest degree of polymerization of polygalacturonic acids which can be precipitated by calcium is also not known. It should be possible to define the lower limit of polymerization according to the possibility of precipitation under well-defined circumstances as has been done for fruit pectins by HINTON (213). According to HINTON the most important feature of fruit pectins is that they are precipitated in a 50% (v/v) acetone milieu in constant yield after manifold repeated precipitation. He preferred precipitation in acetone to precipitation in ethanol since the former treatment gives a firmer filamentous precipitate. When using ethanol, a somewhat greater strength (70%) should be used. Nevertheless, dependent on source, extraction method and other conditions (even after repeated precipitation) some non-uronide may occur in the precipitates. For this reason KERTESZ (259) has suggested that the amount of polyuronides should always be determined in calcium pectate precipitates; the same should also be done in pectic substances precipitated in ethanol or acetone.

After determination of the polygalacturonide content and its degree of esterification, the degree of purity of the purified precipitate can be calculated:

$$\text{Degree of purity} = \frac{(176.x + 190.y).100}{P} \%$$

P = number of grams analyzed
x = number of equivalents of free carboxyl groups in P
y = number of equivalents of esterified carboxyl groups in P

Determination and characterization

Quantitative estimation by gravimetric methods

For reasons mentioned above, objections may be made to the results of gravimetric determinations based upon precipitation of pectic substances by ethanol or acetone, since it is difficult to accomplish complete separation between pectic substances and physically admixed ballast materials.

It is to be expected that better results should be obtained by precipitation of pectic substances as pectic acid or as calcium pectate since, according to this method, the precipitates are formed in the absence of ethanol or acetone. The method for the estimation as pectic acid has been given by WICHMANN (516) while the original calcium pectate procedure was developed by CARRÉ and HAYNES (70). NEWBOLD and JOSLYN (351) compared the results of both methods and considered the Carré Haynes procedure to be superior for the estimation of a crude pectin.

The Wichmann method has not attained wide popularity, whereas the Carré and Haynes calcium pectate method, with some modifications (259), has become one of the few standard methods. Nevertheless, in later years much evidence has shown that, in many cases, appreciable amounts of admixed ballast materials are entrapped in the meshwork of pectic material which causes erroneously high values when determined as calcium pectate (1, 45, 87, 96, 98, 133, 182).

According to the results of DEUEL ET AL (112) the amount of calcium in calcium pectates is equivalent to the amount of carboxyl groups. Therefore the theoretical yield of calcium pectate precipitate from pure galacturonic anhydride is 110.6 % of the weight taken. Indeed, HINTON (213) obtained a 112 % calcium pectate yield from a pectic acid prepared by saponifying a solution of apple pectin with cold sodium hydroxide, precipitated with hydrochloric acid at pH 2 and washed with acid at this pH and finally washed with 80 % ethanol.

However, in many cases the yield of calcium pectate has shown to be much greater than corresponds with the amount of polygalacturonic acid present.

When, according to NEWBOLD and JOSLYN (351), the pectic acid is described as: (uronide + X) +Y, where X is loosely or tightly bound non-uronide matter and Y is physically admixed non-uronide material, it should be possible to remove the Y-fraction by careful washing. For this reason, CASS (72) has proposed that, prior to calcium pectate determination, a pectin preparation should be purified by three-fold precipitation in ethanol. However, PEYNAUD (375) needed six-fold precipitation in ethanol 70 % to remove the Y-fraction from pectic substances in peaches.

DOESBURG (133) has performed titrimetrical polyuronide determinations and calcium pectate determinations in apple extracts which had been purified by one-fold precipitation in acetone 50 %. Such extracts were made during different stages of maturation and ripening of the fruits, by boiling suspensions of the finely milled tissue (peeled and cored) for ½ hour after adjustment to pH 2.6 by addition of some 25 % HCl. As can be seen from Fig. 32, during the earlier stages of development the amount of polyuronide (calculated as pectic acid) was exceeded strongly by the calcium pectate yield which may be due to the presence of solubilized starch. Similar results have been obtained for pears by DAVIGNON (97) and, for apples, by LAWRENCE and GROVE (280). About 10 weeks after the starch in the fruits had disappeared (as controlled by iodine tests) the calcium pectate yield approximated to the theoretical ratio to the polyuronide content. In later stages of fruit development, the calcium pectate yield increased again (Fig. 32).

Similar results have been obtained with two other apple varieties. It may be that the contamination of calcium pectate during senescence of fruits has to be attributed to formation of polyoses, as has been found in ripe pears by JERMYN and ISHERWOOD (235). It is unlikely that the high calcium pectate yields have been caused by the presence of a chemically bound X-fraction, since the jellying power of the extracts was highly correlated with the results of polyuronide determinations (Fig. 32).

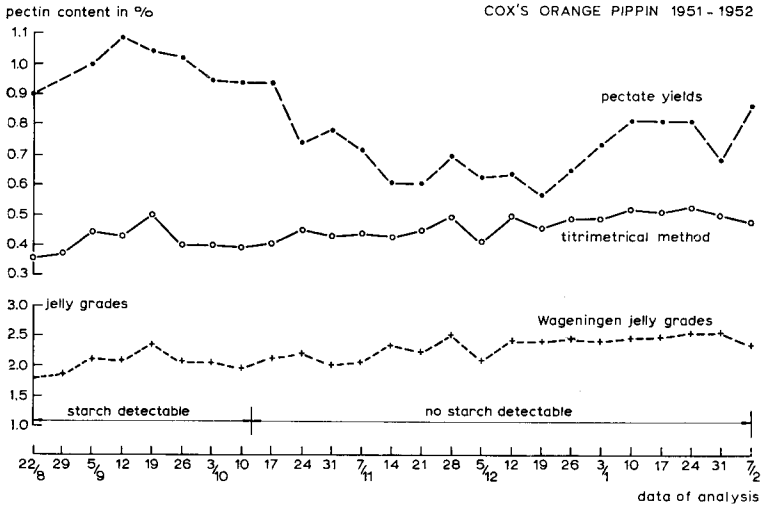


FIG. 32. Results of Ca-pectate and titrimetrical pectin determinations in extracts from Cox's Orange Pippin apples during development and maturation in relation to the jellying power of these extracts (Doesburg, 133).

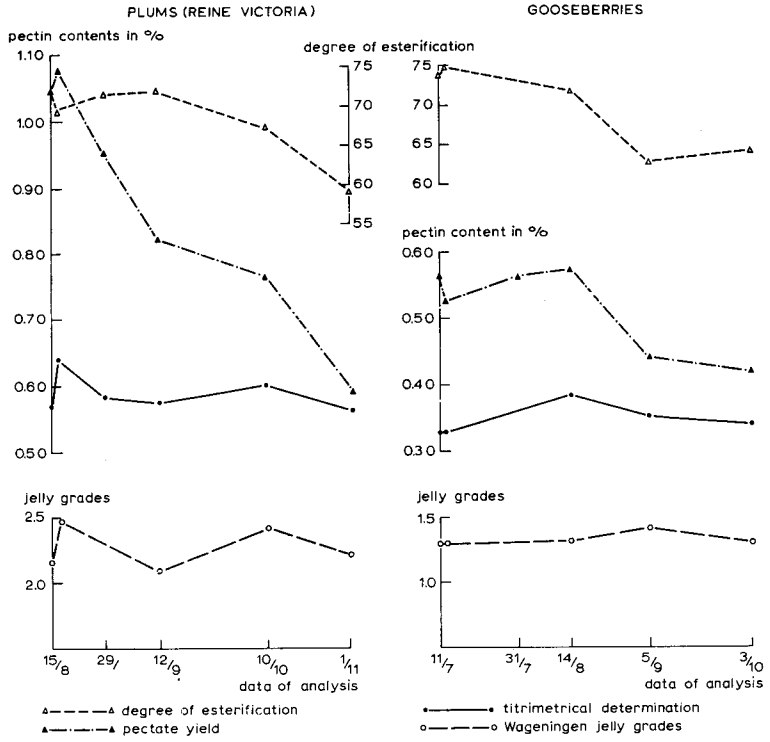


FIG. 33. Results of Ca-pectate and titrimetrical determinations of amount and degree of esterification of pectic substances in Reine Victoria plums and gooseberries during freezing and storage at —18°C (Doesburg, 133).

As has been discussed in Chapter 2 it has to be expected that the jellying power will be influenced by non-uronide materials attached to the polygalacturonic chains.

Similar observations on the discrepancy between uronide content, calcium pectate yield and jellying power have been made by DOESBURG (133) when analyzing extracts from frozen Reine Victoria plums and gooseberries stored at −18 °C. The results are shown in Fig. 33.

The decrease of pectate yields in extracts from stored frozen fruits may be caused by enzymatic breakdown of contaminating polyoses in the fruits. The occurrence of such enzymes in fruits has been reported by ROELOFSEN (407). The decrease of degree of esterification has to be attributed to a slight activity of the enzyme pectin methylesterase (PME) in the frozen fruits.

From the data mentioned above there is no doubt that application of the calcium pectate method should be performed only after careful purication of the solutions of pectic substances.

It is questionable what value has to be given to the numerous results in literature, which have been produced by so many modifications. According to JAKOVLIV (231) these results still have some comparative value and are not only of historical interest; however, even if one agrees with this point of view, it will be necessary to view these calcium pectate values critically.

Nevertheless DESCHREIDER and VAN DEN DRIESSCHE (105), when comparing the applicability of titrimetrical and calcium pectate method to jam analysis, preferred the calcium pectate method. The solutions from which the pectin should be separated before titration are difficult to filter (caused by their high sugar content) and the volumes of liquid are too large to allow the use of a centrifuge.

According to his own experience the author agrees that the purification of pectins from jams is rather tedious. However, GERSONS and DOESBURG (178) compared the results of both methods of analysis on nineteen samples of jam and found again a much higher pectate yield (after correction for the calcium content) than with the titrimetrical method. DAVIGNON (96) reported good agreement between the quantity of uronides calculated from calcium content of the calcium pectate yield and the results of titrimetrical and colorimetric estimations of pectinic acids. From the results of TÄUFEL and BOCK (474) it may be concluded that further investigation of the calcium/uronide ratio is needed, since the amount of calcium in the calcium precipitate is influenced by washing and enclosed impurities.

Quantitative estimation by decarboxylation methods

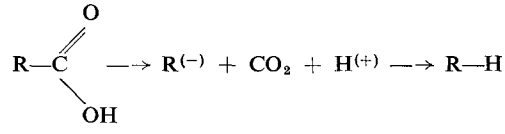
According to the foregoing definitions, the determination of polygalacturonide content of preparations of pectic substances is most important. Further, it is of great importance to determine the degree of esterification and degree of polymerization, since the behaviour is strongly influenced by these properties. An additional point of interest, especially in beet pectins, is the acetyl content which affects the jellying power.

The determination of polygalacturonic acid content has to be carried out on preparations of pectic substances from which the lower polygalacturonic acids, having no colloidal properties, have been removed. This can be done by precipitation with ethanol (70%) or acetone (50%) as described earlier. At the same time, other impurities which may interfere in the determinations, are removed.

Determination of uronide content by decarboxylation of uronic acids by boiling with 12% hydrochloric acid, according to the principles given by LEFÈVRE-TOLLENS (281),

has found wide application. The mechanism of this decarboxylation reactions has been studied by ZWEIFEL (529).

The production of CO_2 from carboxylic acids by boiling with strong acids is often described as an ionic reaction:



The decarboxylation reaction is not specific for D-galacturonic acid.

Mannuronic and glucuronic acid produce about the same amount of CO_2, but much more slowly than does D-galacturonic acid. The difference in reaction velocity of monomer and polymer hexuronic acids is very small since glycosidic bonds are easily hydrolyzed during boiling in strongly acid milieu.

The reaction constants of some hexuronic acids during boiling in 20.2 % HCl at 110° C are given in Table 4 (529).

TABLE 4. *Reaction constants of decarboxylation of uronic acids in 20.2 % HCl at 110°C (529).*

Acids	*Reaction constant K(sec.$^{-1}$)*
D-galacturonic acid	2, 187.10^{-4}
Polygalacturonic acid	2, 160.10^{-4}
D-mannuronic acid	1, 670.10^{-4}
Polymannuronic acid	1, 466.10^{-4}
D-glucuronic acid	1, 648.10^{-4}

The production of CO_2 from ketonic acids is usually rather slow. The decarboxylation of ascorbic acid proceeds rather rapidly.

For the determination of uronic acid content by decarboxylation, the modifications of WHISTLER, MARTIN and HARRIS (511), MCCREADY, SWENSON and MACLAY (321) and the micromethod of TRACEY (478) are often used.

According to KERTESZ (259) the modification of WHISTLER ET AL (511) is to be preferred when analyzing materials rich in unknown carbohydrates, since the curves obtained by this procedure are enabling a correction for the non-uronide carbon dioxide. This should be of particular interest for the analysis of ethanol-insoluble solids of plant tissues without foregoing extraction of pectic substances. According to JOSLYN (245), studies have been made on the kinetics of decarboxylation of uronic acids and their derivatives and of cellulose derivatives, but no similar studies have been made on sugar-free fruit tissue preparations (e.g. alcohol-insoluble solids). For this reason, the results of decarboxylation of ethanol-insoluble solids as a measure of total uronides are unreliable. Until no more data are available on the occurrence and amount of substances liable to decarboxylation in plant tissues, the application of these methods must be confined to extracted pectic substances, of which it can be safely assumed that the greater part of uronide content is formed by polygalacturonic acids.

The simultaneous determination of uronide and methoxyl contents, can be carried out with the method of VOLLMERT (497) in which hydriodic acid is used for the decarboxylation. When taking into account that, with this method the carbon dioxide yield is 7.3% below the theoretical value, in the experience of the author, good agreement was obtained with the results of other decarboxylation or titration methods on pectin preparations.

Quantitative determination by colorimetric methods

Colorimetric methods are used for the determination of uronide content. As compared with the method based on anthrone (197, 198), naphtharesorcinol or dinitrobenzoic acid (58), the carbazole method of DISCHE (122) has found most wide application. MCCOMB and MCCREADY (318) and MCCREADY and MCCOMB (324) modified this method by using a sequestering agent (Versene) and a commercial enzyme preparation for the extraction of total pectic substances in fruits.

The reaction mechanism of the carbazole reaction has been elucidated by STUTZ and DEUEL (467) and STUTZ (466). The reaction compound formed was identified as 5-formylpyroracemic acid.

AHMED and SCOTT (1) compared the results of calcium pectate method (259), decarboxylation method (321) and carbazole method when analyzing hydrochloric acid extracts of alcohol-insoluble solids from various plant materials and pectin preparations. The results, calculated as a percentage of alcohol-insoluble solids, are shown in Table 5. For a proper comparison the calcium pectate yields have been corrected for their calcium contents.

TABLE 5. *Comparison of results of calcium pectate method, decarboxylation method and carbazole method for the determination of pectic substances in various materials* (1).

Materials analyzed	Calcium pectate method	Decarboxylation method	Carbazole method
Citrus pectin	76.76 %	73.44 %	81.23 %
Citrus pectin	75.40	75.56	83.81
Blackberry	4.21	3.64	3.76
Blueberry	5.80	3.85	3.06
Sweet potato	2.43	11.04	10.14
Tomato	3.98	2.72	2.89

When dealing with the fruits and sweet potatoes, the spectrophotometric method is in much closer agreement with the decarboxylation procedure. In the case of citrus pectin preparations the calcium pectate method has given the same results as the decarboxylation method.

Determination of methoxyl groups

The determination of methoxyl groups is mostly carried out in accordance with the principles given by ZEISEL for refluxing pectinic acids with hydriodic acid which causes the formation of methyl iodide. This may be determined gravimetrically as silver iodide or volumetrically as the iodate. The semimicro determination described by CLARK (79, 80, 259) has shown to be very useful.

By use of the Zeisel procedure, volatile iodides are produced also from adsorbed

ethanol or ether-bound OCH_3 groups. Therefore precautions have to be taken to ensure ethanol is absent (e.g. by precipitation with acetone). Application of these determinations to purified plant tissues (e.g. washed with acetone) may give rise to high apparent methoxyl yields, as a result of the presence of ether-bound methoxyl groups in lignins occurring in small amounts in edible plant tissues (31, 229).

TIBENSKÝ, ROSÍK and ZITKO (476) published a rapid method for the determination of the degree of esterification. This method is based on the determination of the amounts of bound Cu^{2+} ions before and after deesterification. The estimated value for the degree of esterification is not influenced by the presence of acetyl groups.

Combination of quantitative decarboxylation method and determination of methoxyl groups

It has already been mentioned that VOLLMERT (497) described a method for determination of uronide and methoxyl contents by using hydriodic acid also for the decarboxylation. In the experience of the author, good results can be obtained when the decarboxylation procedure with hydriodic acid is combined with the modification for estimation of methoxyl groups due to CLARK.

A combination of modified methods of VOLLMERT (225, 497) and CLARK (79, 80, 259) for the estimation of uronic acid and methoxyl contents in preparations of pectic substances is described hereafter. The apparatus needed for these determinations is shown in Fig. 34.

The nitrogen, which is used as the carrier gas for the evolved carbon dioxide passes first through a safety flask A and then through an alkaline solution of pyrogallol B in which the inlet tube is drawn to an orifice to produce fine bubbles. The gas then passes through two absorption towers C filled with soda lime and a second safety flask D and then enters the 100 ml reaction flask R.

To prevent the baking of small bits of the sample which may be splashed against the side, the flask R is placed in an oil bath so that the oil level is 3–4 mm lower than the liquid level in the flask R.

A small funnel is placed upon the flask R for the addition of the hydriodic acid to the contents

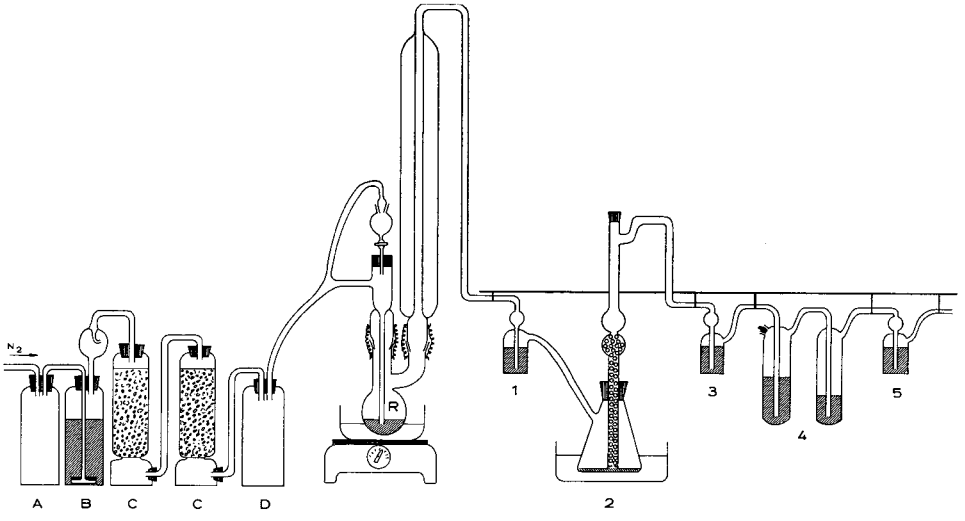


FIG. 34. Apparatus for simultaneous determination of uronide and methoxyl content of pectic substances according to the modified method of Vollmert (Doesburg, 140).

of R; when the cock between the funnel and R is opened, the carrier gas passes through this funnel.

From R, the nitrogen passes through a reflux condenser into vessel No. 1 which contains 7.5 ml of 1N potassium iodide.

In the next absorption tower No. 2, described by HOTTENROTH (225) and slightly modified as shown in Fig. 34, the evolved carbon dioxide is absorbed in 7.5 ml of 0.2N barium hydroxide solution. The tower is filled with small glass balls; during the determination, the upper level of the barium hydroxide solution is raised by the nitrogen flow to the upper level of glass balls.

Vessel No. 3 is filled with 7.5 ml of the same barium hydroxide solution and is used to control the completeness of absorption of carbon dioxide in the absorption tower and preventing the passage of acetic acid from vessel No. 4 to No. 2.

Vessel No. 4 is filled with 15 ml 10% sodium acetate solution in 96–100% acetic acid with addition of 15 drops iodine free bromine solution; the first part of No. 4 has to contain about 10 ml of this solution, the second part 5 ml.

Vessel No. 5 is filled with 7.5 ml diluted formic acid to prevent the escape of bromine vapours into the laboratory.

To determine the uronic acid and methoxyl contents, ± 100 mg of an ethanol-free preparation of pectic substances is placed into the reaction flask R and weighed accurately. When vessel No. 1 is filled with 7.5 ml of 1N potassium iodide solution it is connected with the reflux condenser and with the absorption tower No. 2 (without barium hydroxide solution); then a rapid flow of nitrogen is started and maintained during 15 min. During this period vessel No. 3 is filled with 7.5 ml of 0.2N barium hydroxide solution and No. 4 with 15 ml 10 % sodium acetate in 96–100 % acetic acid and 15 drops bromine solution; 7.5 ml of 5 % formic acid solution is placed into vessel No. 5. Finally the funnel is filled with 7.5 ml hydriodic acid 57 %.

The stream of nitrogen is now decreased so that, with regular flow, about 4 bubbles/sec pass into vessel No. 1. 7.5 ml of 0.2N barium hydroxide solution is now placed into the absorption tower after opening the upper plug and all the vessels are rapidly connected. By opening the cock upon the reaction flask R, the hydriodic acid is placed into the flask R and finally the oil bath is heated rapidly to 160° C and held at 160°–170° C for one hour.

Thereafter, vessel No. 2 is heated at 40°–50° C for 15 min. by placing it in a hot water bath to pull out last traces of methyl iodide. It is useful to replace the hot water once during this period; excessive heating (> 50° C) may cause some loss of carbon dioxide. Vessel No. 2 is then cooled by immersion in cold water to prevent intake of atmospheric carbon dioxide when disconnecting the parts of the apparatus for titration. During the cooling period, the heating of the reaction flask R is completed.

For the titration, the contents of vessel No. 4 are rinsed quantitatively with 160 ml water into a 300 ml titration flask which contains 7.5 ml of 25 % sodium acetate solution. After addition of 9 drops of 98% formic acid, the flask is rotated till the brown colour due to bromine has disappeared; then 18 drops of formic acid 98 % are added and the solution is allowed to stand for 1 to 2 min. Finally, 2 ml of 4N sulphuric acid and 1.5g potassium iodide are added and the free iodine is titrated with 0.1N sodium thiosulphate; 1 ml of 0.1N thiosulphate equals 0.517 mg of methoxyl (CH_3O). The percentage of methoxyl can be calculated from the total quantity of thiosulphate needed, diminished by the amount needed for a blank titration with exactly the same solution as mentioned above (mostly the value of a blank titration showed to be 0.05 ml of 0.1N thiosulphate).

Vessel No. 2 is disconnected and the glass balls are washed with some distilled water from a siphon (the siphon has to be filled with boiled aqua destillata; the air inlet of the siphon has to be protected by a small tube with soda lime to prevent the entrance of carbon dioxide). The absorption tower is then turned so that the glass balls are placed into the barium hydroxide solution. Finally, the barium hydroxide solution is titrated with 0.1N hydrochloric acid using phenolphtalein as indicator. When B ml of 0.2N barium hydroxide solution and H ml of 0.1N hydrochloric acid have been used for the estimation of the carbon dioxide evolved by P mg of a preparation of pectic substances the polygalacturonic acid percentage C of this preparation can be calculated from:

$$\frac{(2B-H).946}{P} \%$$

Since the properties of the pectinic acids are strongly governed by their degree of esterification it is useful to calculate the degree of esterification E of the polygalacturonic acid from the next equa-

tion in which is A = % methoxyl and C = % polygalacturonic acid in the original sample;

$$E = \frac{A.\ 176.\ 100}{C.31}\ \%$$

Simultaneous determination of pectinic acids and their degree of esterification by volumetric methods

The amount and degree of esterification of pure solubilized pectinic acids can be estimated also by titrimetrical methods. The determination is carried out in two steps: first, the solution of pectinic acids is titrated with alkali in the presence of a suitable indicator. The used number of equivalents A corresponds to an equivalent amount of free carboxyl groups present in the pectinic acid solution. Afterwards, in order to saponify the pectinic acids, an excess of dilute alkali, representing X equivalents, is added to the solution previously titrated; the titration flask has to be stoppered to prevent CO_2 absorption from the air. This saponification, lasting at least 30 minutes, is followed by the addition of an excess of acid solution, representing Y equivalents. Finally the number Z equivalents is obtained by back titration with dilute alkali.

The weight W of the pectinic acids in the titrated amount of solution is calculated by the formula:

$$W = \{176.A + 190(X+Z—Y)\}\ g$$

The degree of esterification E can be calculated from the quotient:

$$E = \frac{100\,(X+Z—Y)}{A+X+Z—Y}\ \%$$

DESCHREIDER and VAN DEN DRIESSCHE (105) use methyl red as indicator. HINTON (213) prefers a mixture of 1 volume each of 0.4% bromothymol blue, 0.4% cresol red and three volumes of 0.4% phenol red and the end point is taken when the red colour, indicating pH 7.5, persists for one half minute. DOESBURG (131) has successfully used phenolphtalein indicator, whereas in the potentiometric method of DEUEL (108) titration to pH 7 is carried out.

For the titration with diluted alkali N/20 sodium hydroxide is normally used; the addition of excess alkali and acid is mostly done with N/10 solutions.

It is clear that the pectinic acid solution has to be free from other materials which may influence the titration results, especially acids and bases, which are partially neutralizing free carboxyl groups. For this reason, the pectinic acids need previously to be precipitated with acetone or ethanol after acidification of the solution to pH 1.5 with hydrochloric acid. The residual acid has to be washed out carefully with acetone or ethanol. The removal of bases can be accomplished also by percolating the solution through ion exchange resins (277).

To avoid the long washing need to purify the pectinic acids from contaminating acids, a modification due to DOESBURG (131) may be used.

According to JAKOVLIV (231), it is to be expected that, after acidification to pH 1.5, partial saponification of pectinic acids will take place during standing at room temperature. As has been shown by DOESBURG (133), a very slight decrease of degree of esterification from ± 84.6 % to 82.8 %, of a highly esterified pectin occurred during 24 hour treatment at pH 1.5.

PEYNAUD (375) has shown that the results of titrimetrical estimations are not influenced by proteins, especially gelatin, when added in the same amount as the quantity of pectinic acids. After additions of gelatin, corresponding with 100% or 150% of the present amount of pectinic acids, DOESBURG (133) reported a slight increase (respectively 4% and 6.4%) in the results for content of pectinic acids, whereas the apparent degree of esterification did not seem to be affected. When analyzing extracts from fruits and vegetables, the ratio of protein to pectinic acid is usually lower than in these trials.

Finally it has to be pointed out that contaminating polysaccharides, which contain uronic acids (13, 66, 67, 527), may increase the apparent results of titrimetrical analyses; the same holds for the decarboxylation methods.

Determination of acetyl contents

For the determination of acetyl content of pectic substances, the methods of PIPPEN, MCCREADY and OWENS (381) and HENGLEIN and VOLLMERT (202) call for mention. According to PIPPEN ET AL (381), the results of both methods are in excellent agreement for the analysis of pectin acetates.

Estimation of degree of polymerization

According to HOTTENROTH (225) the estimation of the degree of polymerization or of the molecular weight is one of the most difficult problems in the analysis of pectic substances.

The methods can be divided into chemical and physical (ultracentrifuge, streaming birefringence, osmotic pressure, viscosity) measurements. The chemical estimation depends on the determination of the number of reducing end groups.

As has been reported already in Chapter 1, the results of various methods do not agree well, even for pectic substances having a low degree of heterogeneity.

The results of estimations of the number of reducing end groups have been shown to be unreliable, since the results of these methods are affected by minute amounts of ballast materials. For high-polymer pectic substances the accuracy is decreased by the very small proportion of reducing end groups.

Viscosimetry has been most frequently used to determine the molecular weight. It has to be taken into account that the viscosity of a solution of pectinic acids depends on molecular weight as well as concentration, degree of esterification, purity, presence of electrolytes, pH, electroviscous effects, etc.

DEUEL and WEBER (109) have eliminated the influence of the degree of esterification by deesterification of the pectinic acids to pectates. When using highly esterified pectinic acids, the saponification with alkali has to be carried out at low temperatures to avoid depolymerization. Since pectates are very sensitive to presence of electrolytes they have to be purified carefully by washing with hydrochloric ethanol and finally with ethanol. The viscosity measurements are done in a 0.05 N sodium hydroxide solution to which 0.02 N sodium oxalate has been added to remove traces of calcium to avoid precipitation of pectates.

CHRISTENSEN (78) has given a method for the calculation of molecular weights from viscosity measurements on commercial high-methoxyl pectins.

SCHNEIDER and BOCK (422) have excluded the influence of hydration, electrolytes etc. by converting the pectinic acids into water-insoluble nitropectins. Acetone was used as the solvent for the viscosity measurements.

Determination of jellying power of high-methoxyl pectins

The jellying power is the most important property of pectins, which has caused their production from various suitable raw materials. Since the determination of jellying power is very important for grading commercial pectins it is not astonishing that this subject has been studied intensively and that various methods have been developed.

The jellying power of pectins is mostly measured by estimation of the strength of gels which have been prepared under accurately described conditions. The results of these grading methods are dependent on the measurement of jelly strength as well on the manner of preparation of the jellies. Since viscosity and jellying power are mainly dependent on molecular weight, it might be expected that jellying power can be estimated from viscosity measurements. However, SWENSON, SCHULTZ and OWENS (471) indicated that a precise estimation of jellying power cannot be obtained from viscosity measurements. Viscosity may be used only for comparison of the jellying power of pectins of like source and history (342).

According to CHRISTENSEN (78) the methods of determination of gel strength can be divided into two large groups.

Group I. The elastic limits of the jellies are exceeded and the jellies ruptured; measurement of breaking strength.

a. Succharipa's jelly disc method (468)
b. Fellers-Claque's penetrometer method (162)
c. Lüers-Lochmüllers 'Pektinometer' method (290)
d. Delaware Jelly Strength Tester by Tarr-Baker (21, 27, 472) and several modifications of the same.

Group II. Deformation of the jellies within the limit of elasticity.

a. Bloom's gelometer (53)
b. Cox and Higby's sag method (88) and its modification by the I.F.T. Committee on Pectin Standardization (228); the Wageningen sag method (125)
c. B.A.R. Jelly Tester (69)
d. Säverborn cylindrical torsion method (416).

Not all these measuring devices and their modifications (28, 242, 337, 341) have attained wide popularity for grading of commercial pectins. During the latest decades the method of LÜERS and LOCHMÜLLER (290) has been used extensively in Germany, the B.A.R. Tester (69) or F.I.R.A.-Tester in Great Britain, the Wageningen method (125) in the Netherlands and the Delaware Tester (21, 472) and the COX and HIGBY sag method (88) in the U.S.A. and many other countries.

Since the modification of the Cox and Higby sag method has been described by the I.F.T. Committee on Pectin Standardization (228) it has to be expected that there will be a growing tendency to use this method for grading of high-methoxyl pectins in several countries.

As has been pointed out before, the method of measurement of jelly strength as well as the recipe and method of preparation of the test jellies is important. Since high-methoxyl pectins are mostly used for the manufacture of high-solids jellies, such jellies are used for grading of high-methoxyl pectins, whereas low-methoxyl pectins mostly are graded by preparing low-solids jellies.

It has already been pointed out that the jellying power of high-methoxyl pectins is described in relation to their sugar carrying power. According to the definition, jelly grade is the proportion of sugar which one part of solid pectin (or pectin extract) is capable of yielding, under prescribed conditions, a jelly with suitable characteristics. For instance, from a pectin with 150 jelly grades in a jelly with 65 % sugar (or soluble solids content of 65 %) the percentage of pectin needed to produce a standard gel is 65/150 %.

According to CHEFTEL and MOCQUARD (77), high-sugar gels are non-elastic since they found a proceeding deformation of these gels till they were ruptured, even when weak forces had been applied. Nevertheless this point of view could not be confirmed by the rheological experiments of HARVEY (194) and by DOESBURG (125).

The instrument used by HARVEY (194) in the examination of the rheological properties was an improved version of the Campbell Jelly Tester (69), known as the F.I.R.A. instrument, working with a torsion blade. He showed the behaviour as exhibited by deflection-load and deflection-time curves to be of the same qualitative character regardless of the presence, absence or type of buffering system, and also apparent with other gels such as those of gelatin and agar. It was apparent that such gels possess both elastic and viscous properties and that their behaviour under shearing stress is a combination of such deformation.

DOESBURG (125) concluded also that high-sugar gels possess elastic properties. When taking the test jellies of the Wageningen grading method out of their containers these jellies showed a sag which after one minute remained constant for 48 hours, provided evaporation of water was prevented.

As shown by OWENS, PORTER and MACLAY (362) there may be a good agreement of the results of torsion and sag measurements. In their experiments, shear modulus was equal to one third of the compression modulus if no change in volume resulted from the strains set up in the gel. OLLIVER (354) found also a constant relationship between the readings from the English B.A.R.-instrument (torsion) and the Ridgelimeter (sag measurement) and presented a formula to calculate the percent sag Ridgelimeter from B.A.R.-tester readings. However, the results of Tarr-Baker breaking strength measurements were not proportional to the results of sag or torsion measurements (362). This relation has been studied by CHRISTENSEN (78) when comparing the results of sag measurements and breaking strength (Tarr-Baker) as influenced by molecular weight. His results are shown in Fig. 35 and indicate that the proportion between 'sag grade value' and 'breaking strength grade value' of citrus and apple pectins is dependent on their molecular weights. This means that it will be impossible to alter the existing methods of grade determination so that the sag method will give grade values which coincide with the values determined by the breaking strength method.

From the results of the experiments of CHRISTENSEN (78) it can be concluded that apple and citrus pectins show the same relationship between molecular weight and their grade values (sag grades or breaking strengths grades). This seems to be in contrast with the remarks of OLSEN (357) who stated that jellies made from citrus pectins are comparatively friable and have little elasticity, whereas jellies made from apple pectins are highly elastic.

After this short survey of methods used to evaluate the jelly strength, attention

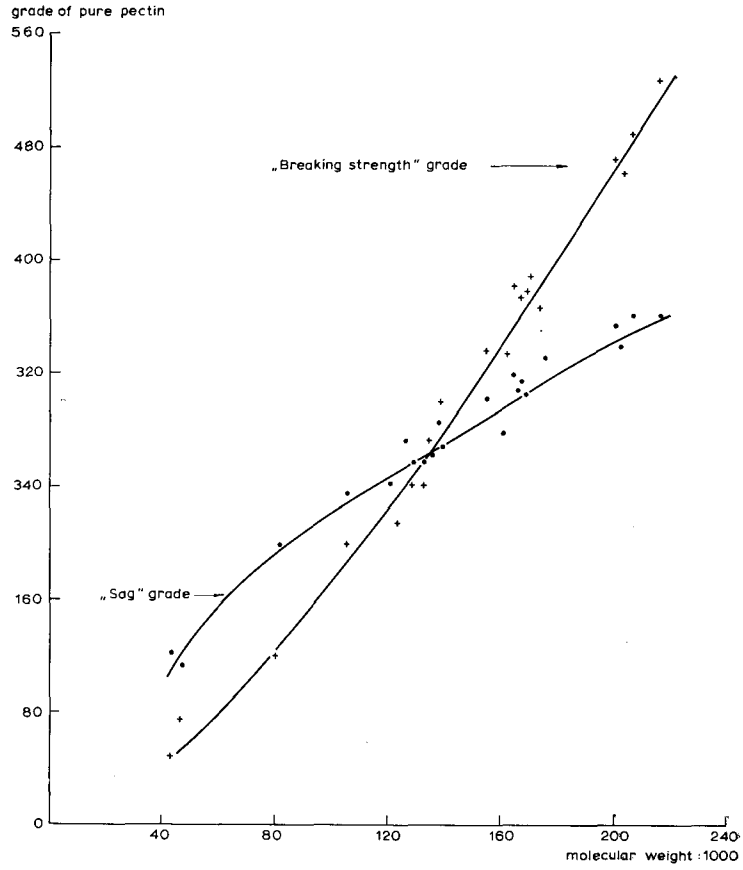


FIG. 35. Different relation of 'breaking strength grades' and 'sag grades' to molecular weight of pectins (Christensen, 78).

must now be turned to the various recipes and procedures for the preparation of test jellies.

As shown in Fig. 15 the jelly strength will be constant and maximal between pH 2.0–2.6 and even higher when the acid has been added just after cooking of the mixture, e.g. when it is present in the glass into which the jelly is poured immediately after cooking ('acid in glass' method). When the acid is present already during boiling ('acid in boil' method) the strength of the finished jellies may be influenced by pre-setting phenomena (see p. 39), which are dependent on pH and ash constituents in the jelly and on the degree of esterification of the pectin used.

In order to prevent pre-setting, in the 'acid in boil' methods it is not possible to use such low pH-values of gels as can be used in the 'acid in glass' methods.

However, even when using pH 3.0 or above, jellies prepared from rapid set pectins by an 'acid in boil' procedure may vary in strength. According to OLLIVER, WADE and DENT (355, 356), this variability, not shown by slow-set pectins, can be overcome by adding surface active agents or fruit juices; by the addition of Teepol, the results should be in line with those obtained by the 'acid in glass' procedure. Nevertheless, it has to be stated here that to the experience of the author the application of such a

Teepol-gel procedure (355) prevented the jellification of some pectin preparations.

PILNIK (377) studied the jellying behaviour of apple pectins with different degrees of esterification in various synthetic and natural media and found a definite relation between setting time and jelly strength according to which jelly strength increases with increasing setting time, whereas under conditions causing rapid setting jelly strength diminishes. Setting time as well as jelly strength were found to pass through an optimum with decreasing degree of esterification in the case of natural media and buffers with calcium, but to increase in the case of buffer media without calcium or excess-acid medium. DOESBURG and GREVERS (143) found a similar relation between the setting times of apple pectins with different degree of esterification and the composition of the media used.

HARVEY (194) emphasizes the importance of avoiding low pH-values when commercial pectins are graded for jellying power, especially when the main commercial use is in the preparation of products, such as jams, where rather high pH-values (3.0–3.5) are involved. Such commercial pectins invariably contain significant proportions of metallic contaminants and clearly, if they are graded at pH-values sufficiently close to 2.0 to suppress most of the ionisation of the free carboxyl groups, then pectins of different metallic contamination may be classed as of similar grade. However, in contrast with this view DOESBURG (125) preferred the use of a low pH which suppresses this influence of metallic contaminations (see also Fig. 17).

According to OLSEN ET AL (359), the optimum pH (see p. 40) can be modified through use of a synthetic juice at pH 3.1, being a mixture of buffer salts and acids which represents the average analysis of a series of strawberry juices. As has been pointed out by BAKER and WOODMANSEE (28) these or other synthetic buffer solutions would be of benefit in grading more nearly to the terms of actual usage in jam and jelly manufacture.

Mostly the soluble solids content of the test jellies is 65%. In England in some methods (355, 371) a final concentration of 70.5% ± 0.5% is used to obtain conditions which are pertaining particularly to jam manufacture in England. The calculation of jelly grade, according to common definition (in a standard 65 percent soluble solids jelly) from English grading results (in a standard jelly with 70 percent soluble solids) was accomplished by OLLIVER (353, 354) after calibration of Ridgelimeter results against a number of jellies prepared according to the English methods.

OLLIVER (353, 354) has mentioned several factors which may materially affect the strength of test jellies and hence the jelly grade.

OLLIVER (353, 354) stressed that the pH appears to be the predominating factor, but rate of initial cooling and temperature of ageing may significantly affect also the strength of pectin jellies and hence the jelly grade. At pH-values above 3.0–3.2 the jellies show increasing sensitivity to changes in ageing temperature irrespective of the type and quantity of buffer salts present. Jellies of 70 % soluble solids content have been found to be much more stable in this connection than those of 65 % soluble solids content. According to the author this difference is related to the fact that the maximum pH of jellification (see page 32) is more elevated in jellies with 70 % soluble solids than with 65 % soluble solids. It has been stressed in Chapter 2 that it is to be expected that setting time is very long in the neighbourhood of the maximum pH of jellying, even at low temperatures (room temperature).

The effect of ageing of 65 percent soluble solids jellies at pH 3.1 from slow-set and rapid-set pectins at room temperature was studied by DOESBURG (125). From the results, shown in Fig. 36, it is clear

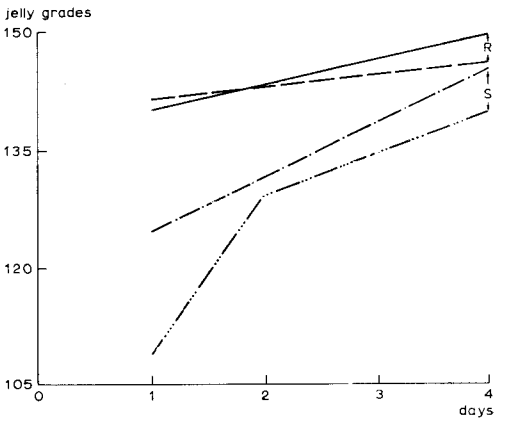


FIG. 36. Jellying power of rapid-set (R) and slow-set (S) pectins measured at different days after boiling. Strength of gels from slow-set pectins increase strongly with time, when jelly grade tests are performed at pH 3.1 (Doesburg, 125).

that jellies are stiffening during a long time and for this reason the calculated jelly grades are increasing after prolonged standing. This effect is much greater with slow-set pectins, since the pH of test jellies was more close to their maximum pH of jellification than for the rapid set pectins with a high degree of esterification. This effect may be another reason for grading pectins with the use of excess of acid at pH 2.0–2.6 (88, 125, 228).

When studying the relation between grading results of two sag methods, the I.F.T. method (228) and the Wageningen method (125), DOESBURG (134) found a different relation between the grading results of both methods for slow-set pectins and rapid-set pectins. Since the recipe of jellies in both methods is the same, but there is some difference in the initial rate of cooling, it has to be expected that these differences of grading results are dependent on this cooling rate as has been mentioned by OLLIVER (353, 354).

From the foregoing it may be clear that it is rather impossible to develop a grading method by which the usefulness of high-methoxyl pectins can be evaluated in different kinds of jellies at various pH-values and manufactured under various conditions. Thus grading methods do not give information on several points of interest to preservers (228). Nevertheless it is of great interest that the jellying power of various types of pectins can be compared under optimal conditions. Since the jelly strength at the higher pH-values of the 'acid in boil' method may be influenced by several factors, the author prefers the 'acid in glass' method which provides an estimation of jelly grade, mainly dependent on the characteristics of the pectin itself (125). As an example of one of such methods the method of the IFT Committee on Pectin Standardization (228) is presented here.

Calculate the weight of pectin to use by dividing 650.0 g by the value of an assumed grade for the pectin. Weigh into a dry container the amount of sugar needed which will be 650.0 g minus the weight (to the nearest gram) of pectin used. Transfer about 20–30 g of the weighed sugar into a dry 150 ml beaker and add the weighed pectin sample. Mix the pectin and sugar thoroughly in the small beaker by stirring with a spatula or glass rod. The sugar should be the finely granulated type made for table use, of which about 75 % is within the band of 35–80 mesh.

Put 410 ml of distilled water into a 3-quart stainless steel sauce pan containing a stainless steel potato masher for stirring. (The sauce pan and stirrer should have been tared previously, on a scale or balance).

The pectin-sugar mixture is now poured into the water all at once, then gentle stirring is started and continued for about 2 minutes. (The object here is to get the sugar-pectin mixture under the

surface of the water as quickly as possible. When hot water is used the sugar dissolves too quickly and the pectin tends to stay on the surface. Splashing when stirring has to be avoided.) Just before the pan is put on the heater or stove any traces of pectin-sugar remaining in the small beaker should be transferred to the jelly batch.

The jelly kettle or sauce pan is then placed on a stove and heated until the contents come to a full rolling boil, stirring being continued during this period. The remaining sugar is added and stirring and heating continued until the sugar is dissolved. Heating is continued until the net weight of the jelly batch is 1015.0 g. The gas or electric stove should be adjusted so that the entire heating time for the jelly is 5–8 minutes!

The stirrer is to be left in the kettle during the cooking and weighing period. If the batch weighs less than 1015 g, distilled water is added in slight excess so that additional boiling will be necessary to reduce the net weight to 1015 g.

After the 1015 g batch is removed from the balance or scale, it is allowed to sit undisturbed on the desk top for one minute, then the pan is tipped so that the contents are nearly ready to overflow, and any foam or scum is quickly skimmed off.

The stirrer is removed and a thermometer is put into the jelly batch. While the kettle is tipped the batch is stirred gently with the thermometer until the temperature reaches exactly 95°C. The batch is then poured quickly into three previously prepared Ridgelimeter glasses, each containing 2 ml of tartaric acid solution. The acid solution should be made by dissolving 48.80 g of tartaric acid crystals in distilled water and making up to a total volume of 100.0 ml in a volumetric flask.

The Ridgelimeter glasses are Hazel-Atlas No. 85 tumblers which have been ground down so that the inside height is exactly 3.125 inches. Scotch drafting tape or Mystik masking tape, $\frac{3}{4}$ inch wide and $9\frac{1}{2}$ inches long is used to make sideboards on each glass (see Fig. 37).

The strip should cover the top $\frac{1}{4}$ inch of the glass and must extend $\frac{1}{2}$ inch above the glass. Squeezing the tape against the glass, especially under the flange near the top of the glass, will insure a tight seal which will not leak hot jelly.

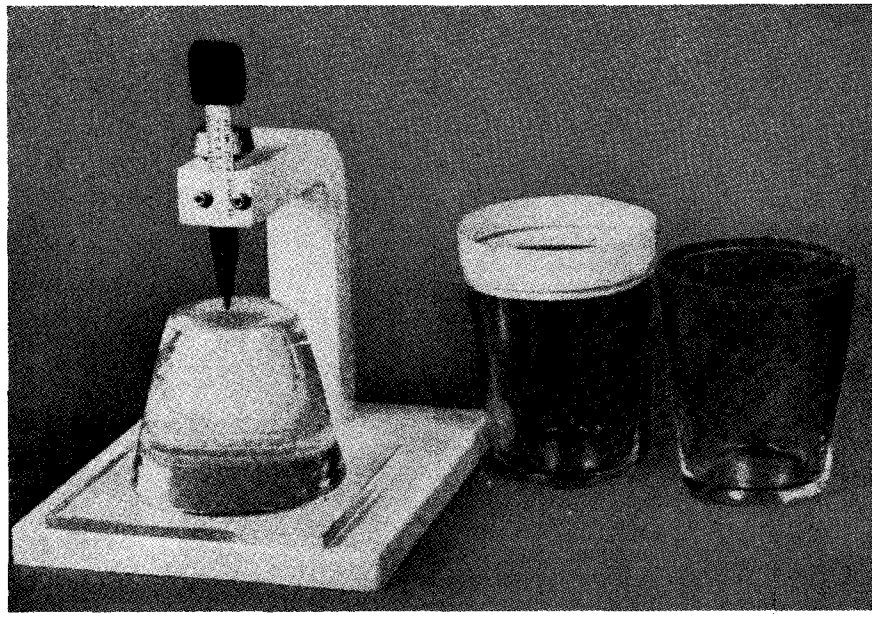


FIG. 37. The Exchange Ridgelimeter, used for determining the firmness of pectin test-jellies. The specially calibrated 'Ridgelimeter Glasses' are shown, with and without the tape 'sideboards' (IFT Committee Pectin Standardization, 228).

During pouring, a glass rod should be in only one of the jelly glasses. When the jelly batch has cooled to 95° C it is poured in the first glass as rapidly as is consistent with reasonably accurate filling, the jelly being stirred vigorously with the glass rod only during filling, but no longer. The glass rod is put into the second glass, pouring is as done before, and so on for the three glasses. It is best to pour very rapidly until the glass is filled part way up the sideboards, then to pour more slowly so that the glass can be filled completely full to the point of overflowing.

About 15 minutes after the glasses are filled they are covered with their regular metal lids which can be fitted snugly over the 'sideboards'. The jellies should be stored 20–24 hours at 25° C ± 3.0° C

After this storage period, the lids are removed from the glasses and the tape strips torn off. A tightly stretched wire, clean and wetted (cheese cutter furnished with the Ridgelimeter) is carefully drawn across the top of the glass while the latter is held upright and is turned slowly part way around, so that a smooth cut is made to remove the layer of jelly projecting above the top of the glass. The detached top layer is carefully removed and discarded.

The jelly is turned out of glass into an inverted position on a plate glass square delivered with the Ridgelimeter. This is accomplished by holding the glass tilted at about a 45° angle while the point of spatula is inserted between the top of the jelly and the glass, to start the separation of the jelly from the glass. The jelly should pull away from the glass while the latter is rotated slowly, without further aid from a spatula. The glass is quickly and carefully inverted just above the glass square in such a way that the jelly slides out and stands upright near the center of the glass plate. Do not drop the jelly on to the plate!

A stopwatch is started as soon as the jelly is on the glass plate. If the jelly leans slightly to one side, this usually can be corrected by gently tilting the glass plate away from the direction in which the jelly leans. The plate and jelly should now be placed carefully on the base of the Ridgelimeter so that the jelly is centered under the micrometer screw which then should be screwed down near to the surface of the jelly. (The Ridgelimeter should be used only on a level desk or table).

Two minutes after the stopwatch was started, the point of the micrometer screw is brought just into contact with the top jelly surface. Illumination should be arranged so that contact of the micrometer tip with the jelly surface can be observed easily. The lowest line on the vertical scale beyond which the lower edge of the circular micrometer head has passed, is the per cent and the number on the micrometer head nearest the vertical scale denotes the tenth of a per cent sag. The Ridgelimeter reading is recorded only to the nearest 0.1 %. When Ridgelimeter readings on different glasses from the same jelly batch differ more than 0.6, the batch should be remade.

The relationship between 'assumed grade' and the factor to obtain 'true grade' is represented by a very slightly S-shaped curve (Fig. 38). In the absence of this curve, the nearly linear relationship allows an approximation by dividing the per cent sag as read on the Ridgelimeter by 23.5 and subtracting this value from 2.0. When multiplying the 'assumed grade' of the test pectin by this latter factor, the approximate 'true grade' is obtained.

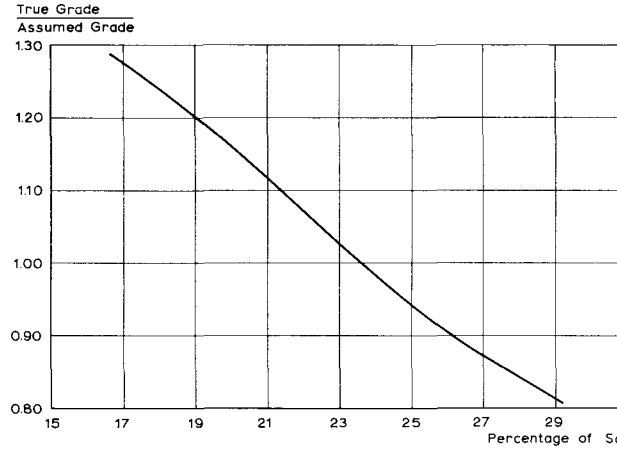


FIG. 38.
Relation between ratio 'true grades/assumed grades' and per cent of sag (Cox and Higby, 88).

Example: When the 'assumed grade' of the pectin was estimated 150, then 650/150 or 4.33 g would be the weight of pectin to use and 650 — 4 = 646 g be the amount of sugar to use. When a sag of 22.0 % was found the 'true grades' would be (2.0 — 0.936) × 150 = 160 grades (recorded only to the nearest grade).

With a curve of the type shown in Fig. 38, jellies which are as much as 20 per cent above or below standard firmness can be graded with accuracy.

According to PILNIK (377) this curve can be used for jellies, made from different pectins and different media. The relation between pectin concentration and elasticity of gels was found to be not dependent on pectin preparation or the media used.

When the soluble solids content of the test jellies is as much as 1.0 unit from 65 % at 20° C, serious errors must have entered into the method of making the jellies. A spread of ± 1.0 % soluble solids can mean an error of ± 3–4 % in jelly grade, a larger error than is involved with ordinary use of the Ridgelimeter.

The acidity and soluble solids may vary from top to bottom of a glass of jelly, regardless of whether or not acid was in the batch or in the glass. However, these variations are too small to be of consequence when the pH is considerably below 3.0 as in this method.

Grading of low-methoxyl pectins

As it has been discussed in Chapter 2, the strength of jellies of low-methoxyl pectins is influenced not only by their pectin content and pH, but also by the calcium to pectin ratio. The optimal calcium to pectin ratio is dependent on the manner of preparation of the low-methoxyl pectins and on their methoxyl content. Moreover, the low-methoxyl pectins may be used in high-solids jellies as well as low-solids products. As compared with high-methoxyl pectins it will be more difficult (or impossible) to develop a grading method for low-methoxyl pectins by which the usefulness under strongly varying practical conditions can be evaluated.

According to HILLS, WHITE and BAKER (209) gel tests with low-methoxyl pectins can be performed in 65% sugar jellies, but the results will give little information on the behaviour of these pectins in low-solids gels. For this reason BAKER ET AL (209) have carried out grading tests in gels with a sugar content of 35%, pH-values ranging from 2.9 to 3.6 and addition of a constant amount of Ca $(H_2PO_4)_2$, sodium citrate and citric acid. Jellies are prepared with varying amounts of pectin. The jelly grade was described as the proportion of water, which one part of solid pectin is capable of turning, under conditions mentioned above, into a standard jelly (firmness corresponding with a breaking strength at 50 cm waterpressure with the Delaware tester (21).).

Rather recently LANGE, BOCK and TÄUFEL (278) developed a modification of this method. First, 35% sugar jellies are made with varying amounts of calcium and citric acid per gram of low-methoxyl pectin. After determination of the optimal calcium and citric acid amounts the water carrying power is determined by the preparation of jellies with varying amounts of pectin. The jelly grade is defined as mentioned above.

A method used for grading low-ester pectins was published by JOSEPH (241, 259).

Since pH, calcium and solids requirements and relationships are different for various low-methoxyl pectins, these data are mostly given by the respective manufacturers, who have developed also tests for standardization. When such requirements are unknown it will be necessary to determine the jellying power according to such laborious methods as developed by LANGE, BOCK and TÄUFEL (278). It may be clear that for this purpose the gel strength can be measured by breaking strength methods

(21, 290) as well as with methods measuring the deformation of jellies without exceeding the limits of elasticity (69, 88, 125, 228, 241).

Determination of setting time or temperature of high-methoxyl pectins

In Chapter 2 it has been mentioned that gels prepared from high-methoxyl pectins may show greatly varying setting times or setting temperatures. In jellies with the same solids contents these characteristics are strongly dependent on the pH, whereas the setting time of high-methoxyl pectins with a relatively low degree of esterification (50–60%) is influenced also by the amount and constituents of ash.

The results of application of high-methoxyl pectins under various practical conditions are strongly influenced by their setting times. Nevertheless little or no work has been done on standardization of these characteristics. As shown by DOESBURG (125) the setting time of gels at pH 2.5 prepared from commercial high-methoxyl pectins, designated as 'rapid-set' varied from 1–33 minutes when the jellies were cooled rapidly to 80° C. For slow-set pectins the setting time always exceeded 80 minutes.

Methods to determine the setting time have been published by PILNIK (377), JOSEPH and BAIER (242), OLSEN ET AL (359) and DOESBURG (125), whereas the determination of setting temperature can be performed according to the principles given by HINTON (215) and DOESBURG and GREVERS (143).

Chapter 4

Pectic enzymes

The occurrence and action of pectic enzymes is important in many respects, in living plants as well as in preserved products. Addition of pectic enzymes to fruit juices is employed for their clarification.

There are some limited observations on the formation of pectic substances derived through the incorporation of various isotope-tagged compounds and groupings into pectic substances (119, 260) but, as stated by KERTESZ (260) in his discussion on this topic, we know little about the mechanism of formation of pectic substances and nothing about the enzymes which are involved in their syntheses, especially in the polymer formation.

The situation is quite different in regard to the action of enzymes upon pectic substances. The literature on these enzymes has been reviewed by JOSLYN and PHAFF (247, 248), DEMAIN and PHAFF (103), DEUEL and STUTZ (119) and REID (401).

Two main groups of pectic enzymes have been described:

1. *depolymerizing enzymes* which split the α–1,4 glycosidic bonds in pectic acid and pectinic acids.

   These enzymes have been designated polygalacturonases (synonyms: pectinases, pectolases, pectin glycosidases, pectin depolymerases, etc.) when they are hydrolyzing these bonds. Another enzyme causing a trans-elimination mechanism, similar to that previously observed for neutral and alkaline degradation of pectinic acids, has been mentioned namely pectin trans-eliminase (5).
2. the *pectinesterases* (synonyms: pectases, pectin methoxylases, pectin demethoxylases, pectin methylesterases) which produce from pectinic acids pectic acids or pectinic acids with a lower degree of esterification while liberating equivalent amounts of methanol and free carboxyl groups.

The pectinesterases occur in the tissues of most higher plants and are produced also by micro-organisms. The polygalacturonases have been reported in higher plants, e.g. tomato (210, 218, 292, 313, 328, 330, 378), avocado (218, 328), cucumbers (34, 35), pears (218, 507, 510), pineapples and medlars (218), carrots (401), and are produced also by micro-organisms (yeasts, bacteria as well as moulds). The enzyme preparations for use in the food industry are of fungal origin (401).

In the past, *protopectinase* has been mentioned often as a distinct enzyme which should produce soluble pectic substances from insoluble protopectin. At present, most workers believe that such a distinct protopectinase does not exist, although the occurrence of non-pectolytic 'macerating enzymes' may be possible (399, 401).

Some evidence about the occurrence of protopectinase has been produced by DAVISON and WILLAMAN (99) in their study on the biochemistry of plant diseases caused by moulds.

At the same time, the softening of fruits from *Mespilus germanica* was investigated by SLOEP (440); from her experiments she concluded that the softening of these fruits during ripening should be

caused by a protopectinase. However, rather recently ROELOFSEN (406) has repeated her work, but he could not reproduce all her results. According to ROELOFSEN the softening of fruits of *Mespilus germanica* is caused by enzymes, but probably this effect is not the result of the action of a distinct protopectinase.

Some other evidence of the occurrence of a protopectinase in pears has been produced by WEURMAN (507). However, his results may be unreliable, since they are based upon calcium pectate determinations (see p. 56).

BEAVEN and BROWN (33) postulated the existence of a macerating enzyme produced by *Byssochlamys fulva*, but experiments of REID (398), showed that polygalacturonase as well as pectinesterase are formed by this organism and may account for its macerating action. Macerating enzymes from *Clostridium felsineum* and *Botryospheara ribis* have been shown to contain also polymethyl galacturonase (254) or pectinesterase and polygalacturonases (307).

Since there is no adequate proof of the existence of protopectinase as a distinct enzyme, some workers believe that the solubilization of insoluble protopectin can be carried out by representants from the groups of pectic enzymes mentioned above, as has been postulated already by COLIN and CHAUDUN (84, 85) and by DEUEL and STUTZ (119) and REID (400).

Further, it is possible that solubilization of pectic substances from protopectin, may be due to the action of enzymes which hydrolyze other polysaccharides linked to the insoluble pectic substances in plant tissues as mentioned before (p. 14 and 58) and by SEEGMILLER and JANSEN (428) and REID (399).

As has been pointed out by DEUEL and STUTZ (119), the results of investigations of pectic enzymes are often difficult to interpret since ill-defined pectic substances (285, 291, 423) and impure pectic enzymes have usually been employed.

Up till now, deacetylating pectic enzymes have not been recorded, whereas very little is known about the enzymic decarboxylation, oxidation and other enzymic reactions which are expected to occur. For this reason the following discussion has to be confined to the groups of depolymerizing and deesterifying enzymes.

Depolymerizing enzymes

Since the Second World War, it has been shown that several types of polygalacturonases exist (19, 233, 424, 425). Extensive research has shown that these various types of enzymes call for different structures around the hydrolyzable α–1,4 glycosidic bonds in order to form the active enzyme substrate complex. Such differences in structure may be caused by the presence of free or esterified carboxyl groups (233, 291, 444) or by the position of the glycosidic bond in the polygalacturonic chain, placed at the end of these chains or not. Some polygalacturonases split the glycosidic linkages at random (endo-enzymes), others from one end of the chain molecule (exo-enzymes). In a number of cases it has been shown that the rate of hydrolysis is strongly diminished when oligo-uronides are attacked (100, 101, 291, 292, 328). Further differences have been reported in relation to the extent of degradation, e.g. to polymeric, oligomeric or to monomeric units.

Enzymes which have been thought to cause such a limited degradation of pectinic acids have been reported in yeasts (428), in *Neurospora crassa* by ROBOZ, BARRATT and TATUM (404), in tomatoes by MCCOLLOCH and KERTESZ (312, 314), and in tomato, carrot and *B. mesentericus* by OZAWA and OKAMOTO (365). However, according to the experiments of ROELOFSEN (405), LUH, LEONARD and PHAFF (292) and MCCREADY, MCCOMB and JANSEN (328) these so-called depolymerases (DPs) are able to cause a breakdown to monomers and dimers and there is no reason to introduce

such a distinct group of depolymerases. However, it has to be remembered that, after a rapidly proceeding initial phase, the hydrolysis may take place very slowly, since according to differences in structure a certain number of bonds are split preferentially and a long time is needed to hydrolyze the remaining linkages (444).

Further, anomalous linkages present in the polygalacturonan chains may cause a limited breakdown.

TABLE 6. *Occurrence of the three types of polygalacturonases.*

Type I: Liquefying polygalacturonases	
Aspergillus aureus	*Fusarium moniliforme*
Aspergillus foetidus	*Neurospora crassa*
Aspergillus niger	*Penicillium expansum*
Bacillus mesentericus	*Rhizopus tritici*
Byssochlamys fulva	*Saccharomyces fragilis*
Clostridium felsineum	*Lycopersicum esculentum*
Ceratostomella ulmi	*Verticillium dahliae*
Type II: Liquefying polymethyl-galacturonases	
Aspergillus niger	*Erwinia carotovora*
Bacterium aroideae	*'Hydrolase'*
Botrytis cinerea	*Pythium de Baryanum*
Type III: Saccharifying polygalacturonases	
Aspergillus foetidus	*Clostridium felsineum*
Aspergillus niger	*Daucus carota*
Clostridium butyricum	*Sclerotinia libertiana*

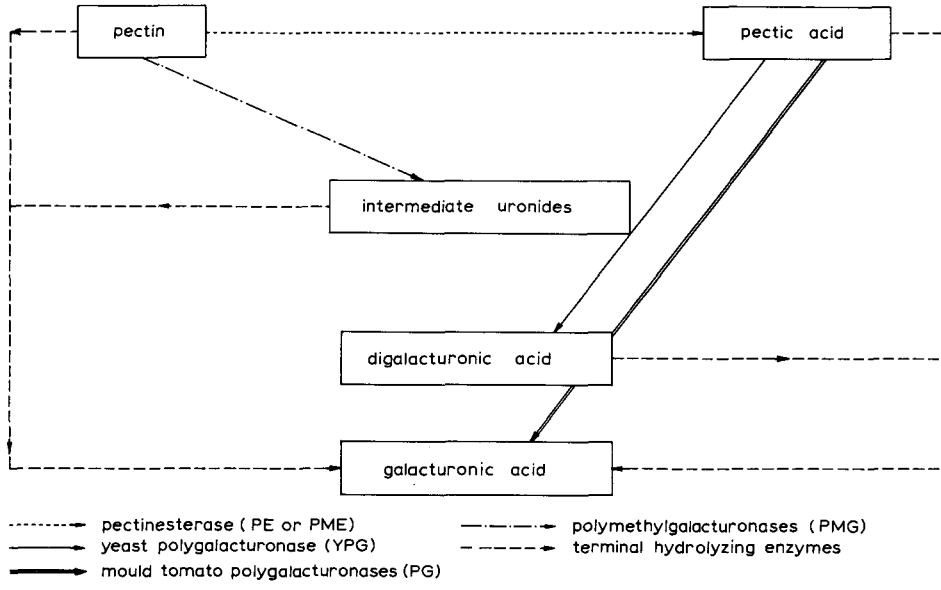


FIG. 39. Possibilities of breakdown of pectic substances by various types of polygalacturonases and pectinesterase (Demain and Phaff, 102).

According to the tentative classification given by DEUEL and STUTZ (119), the *liquefying polygalacturonases* (PGs Type I) are able to split the glycosidic linkages more or less at random and attack preferentially pectinic acids of a low degree of esterification. The *liquefying polymethyl galacturonases* (PGs Type II) preferentially attack pectinic acids of a high degree of esterification, whereas *saccharifying polygalacturonases* (PGs Type III) hydrolyze pectinic acids only from one end of the chain molecule, probably from the non-reducing one. Types I and II are *endoglycosidases* and type III comprise *exoglycosidases*. In Table 6, published by DEUEL and STUTZ (119) an indication is given of the occurrence of various types of enzymes in nature.

Another attempt at a classification of polygalacturonases has been made by DEMAIN and PHAFF (103). Some possibilities of attack of pectinic acids are visualised in Fig. 39.

The polygalacturonases, which have been mentioned above, are hydrolases. However, in a commercial enzyme preparation ALBERSHEIM, NEUKOM and DEUEL (5) found an enzyme which they have named pectin trans-eliminase (PTE), since the reaction caused by this enzyme seems to proceed by a trans-elimination mechanism, similar to that previously observed for neutral and alkaline degradation of pectin. The enzyme attacks only the methyl ester of pectic acid. A study of the breakdown products by ultraviolet absorption, ozonation and reaction with thiobarbituric acid indicated the formation of Δ-4,5 unsaturated galacturonic acid groups, as shown in Fig. 40.

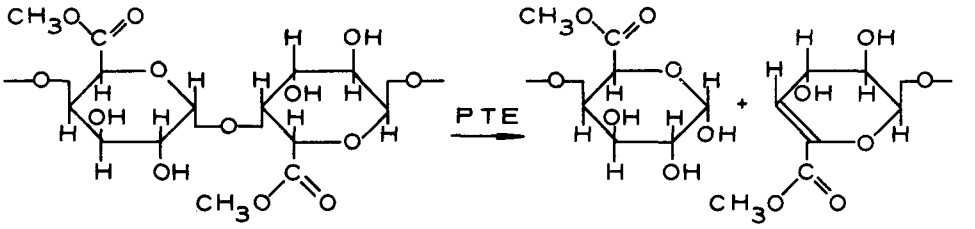


FIG. 40. Mechanism of action of pectin trans-eliminase (Albersheim, Neukon and Deuel, 5).

The optimum pH-values and heat stability of various types of depolymerizing enzymes differ considerably.

The optimum pH of the pectin trans-eliminase showed to be 5.1–5.3; heating during 20 minutes at 60 °C resulted in a loss of 24 % of the original activity, whereas a heat treatment of 1h at 50 °C did not cause any loss of activity (5).

ALBERSHEIM and KILLIAS (6) described a convenient procedure for the purification of fungal pectin trans-eliminase which has a low isoelectric point (pH 3.5).

According to MCCOLLOCH and KERTESZ (313), tomatoes may contain an unusually heat resistant pectolytic factor, showing still 20 % of its original activity when heated 1h at 100 °C in extracts saturated with salt. PILNIK and ROTSCHILD (378), however, could not confirm the presence of such a heat resistant factor in tomatoes.

In most cases, the heat resistance of polygalacturonases has shown to be much less than that of pectinesterases. Complete inactivation takes place by heating to ± 75 °C or lower or 20–30 minutes to 55 or 60 °C, whereas MATUS (304) has indicated that some polygalacturonases are partially inactivated at temperatures ranging from 30–50 °C.

Optimum pH-values of polygalacturonases from moulds have been shown to vary from pH 3.0–5.0. For some polymethyl galacturonases an optimum pH of 5.5–6.0 has been reported (404, 425, 428). SCHUBERT (425) isolated four polygalacturonases from *Aspergillus niger*; a polymethyl galacturonase with an optimum of pH 5.5 and the other three polygalacturonases with an optimum pH of 3.5. MCCLENDON and HESS (309) made a chromatographic comparison of the polygalacturonases in fungal enzyme mixtures.

Polygalacturonases produced by several types of bacteria show generally a high optimum pH-value, ranging from 7.0–8.0 (304, 418).

Pectinesterases

The pectinesterases (PEs), which produce methanol from the esterified groups of pectinic acids, are highly specific enzymes. The PEs from higher plants saponify, almost exclusively, the methyl ester groups of pectic substances. As has been pointed out by SCHILT (418) the name pectin methylesterase (PME) is to be preferred. The enzymes from micro-organisms are often less specific (448). According to MCCOLLOCH and KERTESZ (311), the pectinesterases from alfalfa, oranges and tomatoes are identical (influence of pH, Q_{10}, inactivation temperature) but they have other properties not possessed by the enzymes produced by moulds.

The specificity of orange pectinesterase is shown in Table 7 which has been taken from the review by DEUEL and STUTZ (119).

TABLE 7. *Specificity of orange pectinesterase*

Substrates saponified:
Partial methyl esters of polygalacturonic acid
Protopectin
Substrates not saponified:
Esters of polygalacturonic acid with:
Ethanol (very slowly saponified)
Glycol
Glycerol
Acetic acid
Methyl esters of:
Galacturonic acid
Methyl galacturonoside
Digalacturonic acid (both mono-esters and di-ester)
Methyl digalacturonoside (di-ester)
Trigalacturonic acid (tri-ester)
Tragacanth
Alginic acid
Tartaric acid

The methyl ester of monomeric galacturonic acid and esters of dimers and trimers are not split by the enzymes from higher plants, but in some cases are attacked by the enzymes from micro-organisms, as has been shown by MACDONNELL ET AL (331).

The saponification, caused by pectinesterases from oranges, seems to hydrolyze

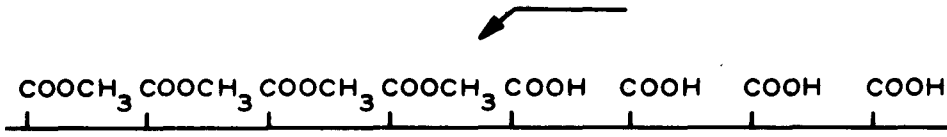


FIG. 41. Mode of saponification by pectinesterase (Solms, 444).

only methyl ester groups adjacent to free carboxyl groups and proceeds linearly along the chain molecule as has been postulated by SCHULTZ ET AL (426) and SPEISER ET AL (451). Therefore pectinic acids which have been partially saponified by pectinesterases may show a blockwise distribution of free carboxyl groups and esterified carboxyl groups as shown in Fig. 41. This manner of distribution of free carboxyl groups in partially enzyme saponified pectinic acids may account for their different behaviour to acids or alkalis as compared with pectinic acid saponified in acid or alkaline milieu. At the same degree of esterification enzyme saponified pectinic acids are more sensitive to coagulation by calcium salts and by acids than other pectinic acids.

According to DEUEL and STUTZ (119) the hypothesis, that pectinesterase splits off successive methoxyl groups along the chain molecule, is supported by several observations.

As long as the amount of attackable ester groups adjacent to free carboxyl groups remains constant the deesterification by the enzyme is a reaction of zero order.

SOLMS and DEUEL (448) showed that the activity of orange pectinesterase is increased when the pectinic acids are pre-saponified by alkali, which causes a random attack of ester groups, resulting in a greater number of ester groups adjacent to free carboxyl groups. Therefore pectinic acids partially pre-saponified by alkali may be expected to have a more or less alternating arrangement of free and esterified carboxyl groups. When highly esterified pectinic acids are partially pre-saponified by alkali the activity of the enzyme increases till by this pre-treatment a degree of esterification of $\pm$ 50 % is reached.

Pectinic acids which have been totally esterified with methanol are very slowly deesterified by the enzyme, since the attack may be possible only after some methoxyl groups have been hydrolyzed by water.

SOLMS and DEUEL (448) have shown that the orange pectinesterase acts very slowly when pectinic acids are partially reduced by $NaBH_4$ which causes the formation of some galactose units inside the chain molecule. The same effect results from the introduction of acetyl groups (418) which are not split off by the enzyme.

The action of pectinesterases can be followed by determination of the increase of free carboxyl groups according to KERTESZ (257) or LINEWEAVER and BALLOU (284), by measurement of the increase in methanol content of the solution, or the increase of the sensitivity of the substrate toward electrolytes (coagulation).

The activity may be influenced by other factors than pH, temperature etc. As shown by JANSEN, MACDONNELL and JANG (233) the action of pectinesterase may be increased by a simultaneous action of polygalacturonases, whereas it was postulated by LINEWEAVER and BALLOU (284) that the action is inhibited by the presence of pectic acid.

LINEWEAVER and BALLOU (284), LINEWEAVER (283) and MACDONNELL, JANSEN and LINEWEAVER (330) showed that the inhibitive action of pectic acid can be decreased by addition of salts; divalent cations have a greater effect than monovalent cations.

The elimination of the inhibitive effect of pectic acid by cations results from a decrease of the negative charge of the pectic acid, by which the positively charged enzyme is released from the pectic acid.

In various plants the pectinesterase is more or less adsorbed by the insoluble solids. According to JOSLYN and PHAFF (248) the juice from alfalfa leaves possesses about 50% of the total pectinesterase activity, the juice of tobacco leaves, tomatoes and oranges respectively 5–10%, 1% and 0% of the total activity in the tissue. KIESER, POLLARD and SISSONS (269) have shown that the enzyme in fresh black currants is associated mainly with the insoluble cell wall tissue, whereas POLLARD and KIESER (382) have postulated the same for apples. The enzyme can be released from the insoluble solids by addition of salts or by increase of pH to 7.5–8.0, by which the charge of the enzyme is altered.

The pH-optimum of pectinesterases produced by moulds and higher plants have been shown to be different. For the enzyme from tomatoes and oranges an optimum pH of $\pm$ 7.5 has been found, for the enzyme from alfalfa these values were 5.6–8.0, dependent on salt concentration. Pectinesterases from moulds have their optimum pH at lower values, ranging from 4–5 (423). However, pectinesterases may show their activity over a wide pH-range, in some cases at pH 2–3, which is important in relation to the behaviour of various plant products in which these enzymes are occurring.

The ease of heat inactivation of pectinesterases is extremely important in relation to the behaviour of citrus juices and concentrates and tomato juices. For this reason the heat resistance has been studied extensively. MACDONNELL ET AL (330) showed that orange pectinesterase is closely associated with the solid particles in the fruit tissue; greater amounts of pulp increase the temperatures and times needed for successful treatment as shown by ROUSE and ATKINS (410) and CHARLEY (74).

The pectinesterases are comparatively heat stable. In raw apple juice 80% of the activity is retained after heating at 68 °C for 40 minutes (382), whereas for successful inactivation in orange juices, heating during 23 sec. at 92 °C was found to be necessary. PILNIK (376) has pointed out that, without holding time, heating to 96 °C is necessary for complete inactivation of the enzyme in orange juices.

Interaction of pectic enzymes and influence of some external factors

In many cases depolymerizing enzymes and pectinesterases are produced by the same organism. According to MACDONNELL, JANSEN and LINEWEAVER (330) the pectinesterase in the flavedo of oranges is not accompanied by other pectic enzymes, whereas WAGGONER and DIMOND (502) showed that the mould *Fusarium oxysporum* produces only pectinesterase when it is grown on a dextrose containing medium. PILNIK and ROTHSCHILD (378) have postulated that the pectinesterase in the juice from tomatoes is free from depolymerizing enzymes.

Methods to free pectinesterase from depolymerizing enzymes or vice versa have been described by SMYTHE, DRAKE and MILLER (442) SCHUBERT (425), ANYAS-WEISZ (9) and MCCOLLOCH and KERTESZ (310).

As mentioned earlier the action of pectic enzymes will be influenced by the presence and action of other pectic enzymes as well by their substrate.

The effect of polymethyl galacturonases will be restricted when pectinic acids with a high degree of esterification are attacked simultaneously by pectinesterases. Otherwhise the depolymerizing effect of polygalacturonases Type I, which hydrolyze (preferentially) pectinic acids with a low degree of esterification, will be promoted by the presence of pectinesterases. This has been shown by SCHUBERT (423) when studying the effectiveness of commercial enzyme preparations for clarification of apple juice.

ALBERSHEIM (3) showed that the pectin trans-eliminase can be inhibited by indol acetic acid, a fact that may be of plant physiological significance.

There are several indications that pectic enzymes may be inhibited by naturally occurring compounds.

WEURMAN (508) was the first to report a thermolabile polygalacturonase inhibiting substance occurring in pears. GROSSMANN (184) found that pectic enzymes of *Fusarium oxysporum f. lycopersici* are inhibited by tannins, and PORTER ET AL (385) reported that the polygalacturonase inhibiting substances from grape leaves (38, 160, 386) could be identified as tannins or tannin-like materials. KIESER, POLLARD and STONE (268) produced evidence that the pectinesterase in black currants is inhibited by phenolic substances. A number of phenolic substances present in cider apple juices, more especially partially oxidized or condensed leuco-anthocyanins, are known to inhibit pectic enzymes, including yeast polygalacturonase and apple pectinesterase (383). BELL ET AL (41a) described the inhibition of pectinolytic and cellulotic enzymes in cucumber flowers by extracts of sericea *(Lespedeza cuneata Don.)*.

KERTESZ (259) has pointed out that pectinesterase from higher plants is unusually resistant to the effect of chemical agents such as formaldehyde, iodine, iodoacetic acid, hydrogen cyanide, mercuric chloride, copper sulphate and other compounds in resonable concentrations. EDWARDS and JOSLYN (151) did not find an inhibiting effect due to cysteine hydrochloride, sodium sulphide and hydrogen peroxide.

Orange pectinesterase is inhibited by relatively high sugar concentrations, as shown by PILNIK (376). CHANG ET AL (72a) showed that 13% sucrose inhibited gelation of papaya puree, resulting from pectinesterase activity. Purified pectinesterase was inhibited at the same concentrations that delayed gelation of the puree. The inhibitory effect was linear with sucrose concentration throughout the range investigated (up to 50% sucrose). The optimum pH (7.5) and the optimum NaCl concentration were not affected by sucrose. Other sugars (glucose, maltose, and corn syrups) were also inhibitory.

The fungal pectinesterases are even more resistant to chemical inactivation. Synthetic detergents of the sodium lauryl sulphate and alkyl aryl sulphonate types have found to be capable of inactivating pectinesterase from higher plants; detergent concentrations which inactivate the plant enzyme do not affect, or only partially inhibit the fungal enzyme. According to MCCOLLOCH and KERTESZ (311) the fungal pectinesterase from the commercial enzyme preparation Pectinol is not inactivated by one hundred times the ratio of an alkyl aryl sulphonate (Nacconol NRSF), which suffices for tomato pectinesterase (11 milligram per cent).

RAHMAN and JOSLYN (390) made experiments on inhibition of the polygalacturonase of purified Pectinol fungal preparations by use of mercurous chloride, sodium fluoride, iodoacetic acid and sulphur dioxide with no apparent inhibition occurring. It has been

postulated by KERTESZ (259) that alkyl aryl sulphonates are much less effective as inhibitors of polygalacturonase. However, STEELE and YANG (455) have stated that the detergent Nacconol NR was found to be an effective inhibitor for the polygalacturonase in Pectinol M in cherry brine at pH 2.6 and in buffer solutions at pH 3.0, 3.6 and 4.0.

Activation by cations of pectinesterase from higher plants has already been mentioned. SCHULTZ (425) has postulated that this effect cannot be regarded as a real activation, but that cations are acting as anti-inhibitors since they are eliminating the inhibiting effect of the formation of the complex of the enzyme and pectic substances. Addition of electrolytes may increase or decrease the enzyme activity of polygalacturonases (119). Experiments of EDWARDS and JOSLYN (151) showed that ascorbic acid exerted no effect on orange pectinesterase activity either at pH 4.0 or 7.0 when used at 0.01 M level, but that it produced a slight increase when present in 0.05 M concentration. A greater activation was found by addition of 0.05 M sodium bisulphite.

Use and production of pectic enzymes

A review of the use of pectic enzymes for industrial, analytical and pharmaceutical purposes has been given by SCHILT (418). Within the scope of this book attention will be paid only to the effect of these enzymes in relation to the technology of fruit and vegetable products.

Pectic enzymes may have considerable deteriorating, as well as beneficial effect, during the production and storage of several of these products. Changes in texture are often related to alterations in pectic substances caused by enzymes, which may have a firming as well as a macerating effect on plant tissues. Various examples of this effect of pectic enzymes will be discussed in Chapter 6.

Pectinesterases may be used for the manufacture of low-methoxyl pectins (209, 343), but they cause a marked heterogeneity of the molecules with respect to degree of esterification as reported by HILLS ET AL (212) and WARD, SWENSON and OWENS (503).

Addition of pectic enzymes of fungal origin has found wide application in fruit juice manufacture. They can be employed when a brilliant clear juice is required. Since soft fruits are not easy to press before a thorough removal of pectic substances has been achieved these fruits are milled and treated with enzymes before pressing.

Details of production of commercial enzyme preparations are rather scanty. As reported before, enzymes of fungal origin are generally used. The enzymes produced by these organisms are most suitable for use in fruit products, since they have a relatively low pH-optimum. Pectinesterase preparations for experimental use are often prepared from orange peel, tomatoes or alfalfa.

For the clarification and pressing of fruit juices, it is desirable to produce preparations of pectolytic enzymes which are balanced in their content of several enzymes, for instance pectinesterases and polygalacturonases. The production of relative amounts of these respective enzymes may vary from strain to strain, with the medium and with cultural conditions, depending upon whether these enzymes are constitutive

or adaptive enzymes. The enzymes have to be suitable for use at elevated temperatures in order to speed up their operation in fruit products.

A survey of micro-organisms which produce pectic enzymes has been given by KERTESZ (259) and MATUS (304). Rather recently BILIMORIA and BHAT reported on the formation of polygalacturonases by yeasts (47).

VITINKA, ROSA and STROS (495) have stressed the significance of the genus *Aspergillus* in relation to production of pectolytic enzyme preparations; according to their review, strains of *A. niger*, *A. flavus*, *A. oryzae*, *A. fumigatus* and *A. wentii* are used for this purpose. REID (400) reported the use of *A. foetidus* Thom and Raper and *A. wentii*, MCCLENDON and HESS (309) mentioned *A. niger* and *Rhizopus tritici*. ENDO and MIURA (157) investigated the production of such enzymes by 250 strains of moulds including plant pathogenic micro-organisms grown on solid media. Forty four of them were found to be capable of clarifying fruit juices. *Conothyrium diplodiella* proved the most active of all for this purpose, hydrolyzing pectinic acids as well as pectic acid; defatted rice bran was found to be the most favourable culture medium, by use of which, production of a significant quantity of the enzyme was observed after 3 to 4 days at 26°C (156).

The organisms are mostly grown on a solid medium. However, it has been found that *Penicillium expansum* was better grown by submerged processes. *A. aureus* could be cultivated successfully by either surface or submerged culture (301). Adequate sterilization of the substrate and of the air stream used for aeration are of great importance. An example of production of pectolytic enzymes from *A. foetidus* has been described by REID (400, 401).

In the production of Filtragol, bran (1000 kg) and water (800 l) were mixed and sterilised in a rotary steriliser and allowed to cool. The bran was then inoculated with a spore suspension of *Aspergillus foetidus* Thom and Raper (50 x 2 l cultures in 5 % molasses), and spread on trays and incubated 2 days at 35° C with suitable control of temperature, humidity and aeration. The bran substrate was then dried in a current of warm air and extracted with ethanol, to remove undesirable odours, and dried and milled.

The form of the final enzyme preparation varies: the whole culture may be dried or it may be extracted with water. With suitable equipment the extract can be concentrated to a stable syrup by low temperature evaporation, or the enzymes in the extract can be precipitated with ethanol or acetone at low temperature (to prevent excessive inactivation), dried and mixed with an inert carrier such as dextrose to a standard activity.

A. niger mycelia, resulting from citric acid production, have been shown to be a suitable source of pectolytic enzymes after washing with cold water, standing 1–2 days and drying at 90°C (301). The wastes of penicillium production also can be used for the manufacture of pectic enzymes (495).

Chapter 5

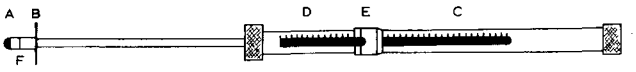
Pectic substances in living fruits

The phase in the life of fruits in which the food industry is most interested, is their maturity. As has been postulated by HULME (227), this phase is essentially one of dying. The object of fruit storage industry is to prolong this dying phase as long as possible.

For these reasons, it is not surprising that most investigators have studied the changes in pectic substances during the periods of harvesting and subsequent storage or processing. The behaviour of pectic substances, which are expected to be the most important cementing materials between the cells, will affect the softening of living and processed fruits. In relation to this, it has already been mentioned that during maturation of fruits a part of the pectic materials is converted into a soluble form.

Relation between pectic substances and firmness of fruits

The firmness of fresh fruits is often measured by use of the Magness-Taylor pressure tester (82, 295, 441) which indicates, in pounds, the pressure required to force a plunger into fruit to a depth of five sixteenth of an inch (Fig. 42).



A = plunger head B = guard disk C = spring housing D = scale
E = moving indicator which shows maximum pressure exerted before puncture
F = line indicating $^{5}/_{16}$ inch distance from top of plunger

FIG. 42.
Magness-Taylor pressure tester.

The pressure testers are now made in two ranges. One that will register 0–10 pounds pressure and another that will register a pressure from 0–35 pounds. There are also two plungers that are in use, the 5/16 inch diameter tip which is mostly used on pears and peaches and the 7/16 inch diameter plunger which is commonly used on apples. On pears the 0–35 pound tester is commonly used for checking fruit prior to ripening whereas the 0–10 pound tester is intended for checking fruits after ripening.

Before making the pressure test with the Magness-Taylor tester a thin slice is removed from the surface of the fruit. Then the fruit may be placed against a solid surface before making the test. The plunger tip is placed against the cut surface and pressure is applied. When the plunger tip has penetrated to a depth of five sixteenth of an inch, indicated by a line on the plunger tip, the pressure reading may be taken from the scale directly. In order to obtain a satisfacting indication of the firmness at least 10–20 apples should be used.

According to SMOCK and NEUBERT (441) the results of pressure readings depend on seasonal variations, the side (blushed or unblushed) of the fruit, fertilization, temperature and moisture contents of fresh fruits.

As postulated by HALLER (188), several cells are squeezed by the plunger, whilst other cells are pushed aside. Therefore the results of pressure tester readings should

be influenced by the size of cells and of intercellular spaces and the thickness of cell walls. ULRICH (482) has stressed that pressure test readings are influenced also by the turgescene of tissues. In relation to this, it should be mentioned that DOESBURG and GREVERS (141) proved that hardness meter readings of raw asparagus greatly depend on turgescence. For these reasons it can be easily understood that not all changes of firmness of fruits during development and ripening will be caused by changes of pectic substances in the fruits.

In Fig. 43 the results are shown of pressure test readings obtained on apples before and after harvest and during storage at 10°C. The pressure tests were made on the unblushed sides of fruits and were carried out on identical samples of fruits by use of a 5/16 inch diameter plunger and a 7/16 inch diameter plunger. In the same lots of fruits, the total content of pectic substances and the amount of soluble pectic materials were determined also (130, 131).

From the results of pressure tester readings it will be noted that there is a gradual decrease of firmness of apples before and after harvest, which is not related, in all cases, with changes in the content of pectic substances or with solubilization of a part of pectic materials. It may be that the rather sudden decrease of pressure tester readings with the 7/16 inch plunger from 25 pounds to 18 pounds is caused by the solubilization of a part of pectic substances in the same period.

The trend of pressure tests readings with a 5/16 inch plunger shows even less rela-

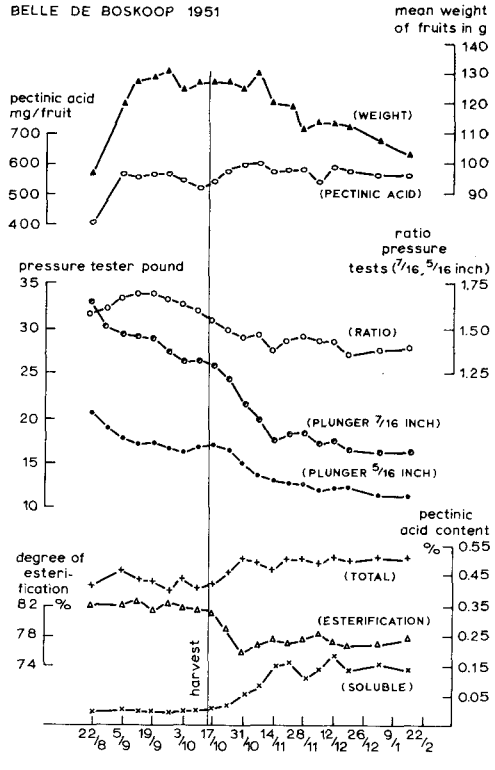


FIG. 43. Pressure test readings, changes of pectic substances and weight of fruits during development, maturation and senescence stage of Belle de Boskoop apples (Doesburg, 140).

tionship with the behaviour of pectic substances in the fruits as was found in several experiments (126, 130, 136). It is clear that the pressure tester measurements with 5/16 and 7/16 inch plungers are not affected in the same manner by changes in the structure and composition of fruits. DOESBURG (126, 130, 136) showed that the ratio of measurements with both plungers always decreases with development and maturation of apples. Before pectin solubilization took place, this ratio was, in most cases, higher than 1.50, while it decreased to a lower value as soon as pectin solubilization occurred (see Fig. 43).

In the stages before harvest, the decrease of pressure tester readings may be caused by increase of size of cells or by the breakdown of starch as has been postulated by WILEY and HENBRIDGE (519), whereas further softening may be due to changes in pectinic acids and hemicelluloses (98). It was postulated by KERTESZ, EUCARE and FOX (265) that firmness of freshly picked apples seems to be closely related to their cellulose content, whereas during ripening the softening should be the result of pectic transformations.

STERLING (458) studied the physical state of cellulose during ripening of peaches and showed that small increases in micellar size and in percentage crystallinity are a function of the maturation process.

In several experiments it has been tried to use pressure tester readings for the determination of the optimal picking date of apples. The use of this instrument was successful only in some instances (481). However, it has been shown that, generally, the indication of such tests, which are based upon phenomena related to the solubilization of pectic substances during maturation of apples and pears, are obtained too late for the determination of the ideal picking date for fruits which have to be stored (128, 136). In order to prevent woolliness of peaches during storage it has been shown that the fruits must be allowed to ripen until the ratio, soluble/insoluble pectic substances, has attained a value $\pm$ 2 before they can be cooled (186).

Changes of amount and concentration of pectic substances during development and ripening of fruits

According to HULME (227), very little work has been carried out on the changes in pectic substances in apples and pears during their early development on the tree. Such investigations have been made on apples by WIDDOWSON (517) and EGGENBERGER (152) and on pears by WEURMAN (507). As summarized by HULME (227), the content of pectic substances is usually higher during the early stages of development of fruits, whereas during the remainder of the period to picking time the contents of total and the small amount of soluble pectic substances fluctuate around a mean value. However, since these results are produced from calcium pectate determinations (507, 517) or from determinations by decarboxylation of pectic substances *in situ* (152) there may be some doubt that this picture needs some correction (see p. 56 and p. 59). The same remarks have to be made about the increase of protopectin content which has been observed in stored apples by use of calcium pectate determinations (487).

The rather constant contents of pectic substances of apples and pears in the period prior to commercial harvest (98, 130, 507, 517), which is shown also in Figs. 32, 43 and 45, is an indication that in this period the growth of fruits is counterbalanced by the formation of pectic substances.

After harvesting of apples, the contents of pectic substances are constant for a long time, as shown by EGGENBERGER (152), DAVIGNON (98) and DOESBURG (131).

The small increase of contents of pectinic acids (Figs. 32 and 43) is caused by the loss of weight of the apples during storage. As cited by HULME (227) in his review on pectic substances in fruits, DZÁMIC (150) reported that some of the domestic varieties grown in Yugoslavia exhibited the same general trend in total amount of pectic substances as mentioned above, but that the variety Kolačarka showed a steady but pronounced fall from harvest to the end of November, followed by a rapid fall. In over-ripe stages, some decrease of total pectinic acid contents of apples is reported by EGGENBERGER (152) and DAVIGNON (98). WOODMANSEE, MCCLENDON and SOMERS (522) proved that the total pectinic acid contents, calculated on a fresh weight basis, decreased significantly from the unripe to over-ripe stages of Stayman apples, whereas in Red Delicious apples these contents decreased, but without significance.

When summarizing the foregoing data it can be said that in most cases the total pectinic acids contents of apples has shown to be reasonably constant from several weeks prior to harvest to the stage of senescence, when a decrease may be found. In some cases (150, 522) a decrease has been reported in earlier stages.

DAVIGNON (98) has also investigated the amount of pectic substances formed during development and maturation of cherries and pears. In cherries, the amount of total pectinic acids per fruit is increasing strongly till about the time the maximum weight of fruit is reached, thereafter this content is decreasing at a rather rapid rate. By contrast with apples, the formation of pectic substances is not in balance with the growth of fruits on the tree, which results in a decrease of the contents during the growing period. The results are shown in Fig. 44. In another season similar results have been obtained.

The results obtained with Passe Crassane pears are plotted in Fig. 45. During de-

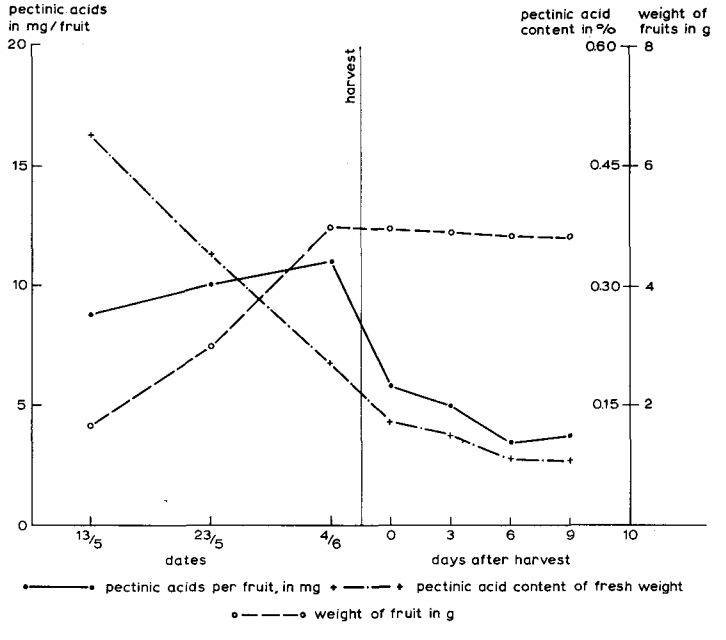


FIG. 44.
Pectic substances and weight of cherries during development and ripening (Davignon, 98).

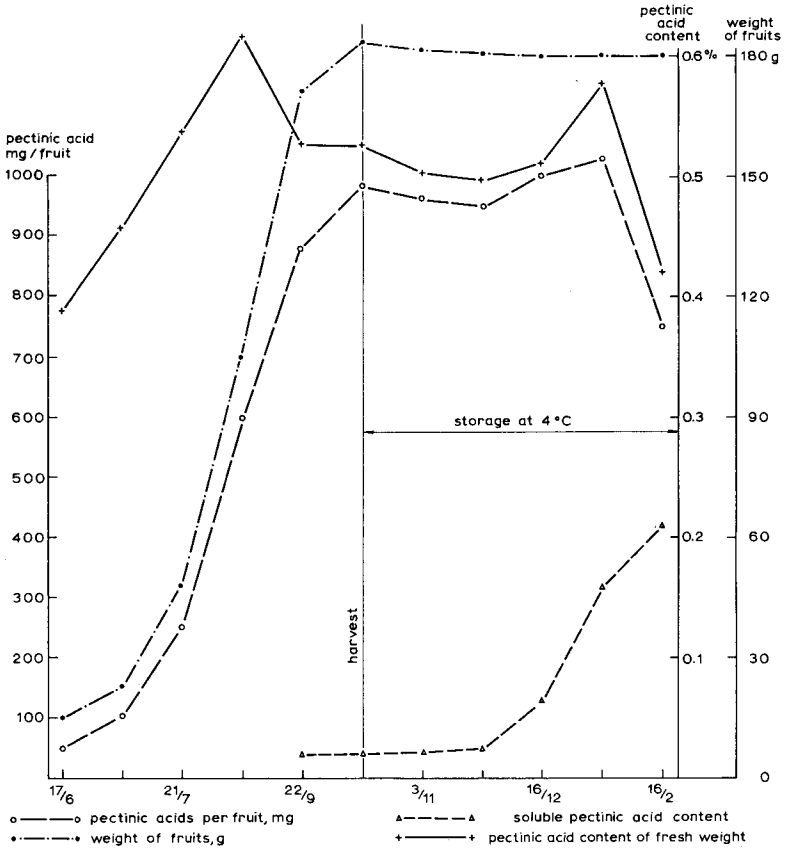


FIG. 45. Pectic substances and weight of Passe Crassane pears during development on the tree and during storage at 4°C after harvest (Davignon, 98).

velopment on the tree the contents of pectic substances increased, reaching a constant level some weeks before harvest. During storage at 4°C, the amount of pectinic acids remained almost constant, followed by a partial breakdown. As shown by ULRICH ET AL (484, 485, 486, 488), it may be expected that solubilization and breakdown of pectinic acids occurs within a rather short period after commercial harvest when a higher storage temperature is used.

WOODMANSEE ET AL (522) investigated the total contents of pectinic acids in tomatoes. It was shown that these contents calculated on fresh weight basis decreased significantly from unripe to over-ripe stages of Valiant tomatoes, but during this period the contents of Brookstone tomatoes were constant.

KERTESZ and MCCOLLOCH (263) found that the pectic constituents in John Baer tomatoes do not show definite trends in either quantity or composition during the period from just before until just after the peak of optimum ripeness for processing. The different influence of ripening at 15°C and 30°C was investigated by FODA (164) and will be discussed later (see p. 93).

SINCLAIR and JOLIFFE (436) followed the seasonal changes in concentration of pectic substances of the peel and the pulp of Valencia oranges during growth and development. With the growth of fruits a strong increase of total and water soluble pectic materials occurred, which was followed by a gradual decrease through the remainder of the season.

Solubilization and changes of degree of esterification of pectic substances, in relation to pectic enzymes

As reported before, by many investigations, much attention has been paid to the changes of pectic substances during ripening of fruits or during storage, especially of apples and pears.

The most important pectic changes arise through the formation of soluble pectinic acids from insoluble protopectin and a decrease of total pectic substances during senescence of fruits. The rate of these pectic changes depends on the kind of fruits and several external factors.

The influence of cold storage and other treatments on pears is studied by ULRICH ET AL (484, 485, 486, 488). It was shown that solubilization and breakdown of pectinic acids in Williams pears was strongly retarded by storing the fruits at 0° C. When after storage at this temperature the fruits were placed during 4 days at 15° C, and subsequently again at 0° C, a part of pectic substances was solubilized during storage at 15° C; this fraction remained constant during subsequent storage at 0° C and no breakdown of pectinic acids occurred. When these fruits were stored finally at 15° C, a rather rapid solubilization and breakdown took place (484, 486).

Pears previously stored at 0° C showed no formation of soluble pectinic acids when they were placed at 18° C in nitrogen atmosphere (488). In waxed pears the solubilization and decrease of pectinic acids is markedly delayed also (485). It has been postulated by ULRICH (483) and ULRICH and MIMAULT (488) that fruit softening seems to be closely linked with respiration. Thus, the similitude of the curves of respiratory activity and formation of soluble pectinic acids, the stimulative effects of temperature on this two phenomena, ethylene and wounding, and other observations lead to this conclusion.

HARTMANN (192) showed that in apricots soluble pectinic acids were formed until the onset of climacteric phase and then decreased. In cherries this decrease of soluble pectic material preceeded the climacteric phase.

DATE and HANSEN (95) studied the behaviour of pectic substances in fruits of three pear varieties during storage at 30°–31° F. Tabulated results show that both protopectin and soluble pectinic acids increased during storage. Since the Ca-pectate method was used, it may be supposed that this apparent increase is caused by the formation of other constituents which are enclosed in the pectate precipitate. When the fruits were ripened at 68–70° F the amount of protopectin decreased and the amount of soluble pectinic acids increased during the early stages of ripening, but decreased during the post ripening period. DATE and HANSEN showed that at the end of the storage period at 30–31° F Bartlett pears failed to soften on removal to higher temperatures for ripening, which was related to a diminishing ability to form soluble pectinic acids.

HANSEN (191) found a distinct relation between decrease of pressure test readings and formation of soluble pectinic acids in pears, which may be promoted by ethylene treatment of fruits.

The solubilization of a part of pectic substances during ripening of fruits is one of the most outstanding features in the behaviour of pectic substances during ripening of fruits. Several investigations have been made of the cause of this effect, which is mostly related to the softening of fruits during ripening. POSTLMAYR, LUH and LEO-

NARD (387) suggested that the firm texture of ripe clingstone peaches may be attributed to a high retention of protopectin in thick intact cell walls, whereas in freestone peaches a large part of the protopectin is converted into water soluble pectinic acids during ripening.

MOGHRABI (338) and FODA (164) found that decrease of protopectin content in tomatoes was connected with softening of fruits.

As reported before, ULRICH (483) and ULRICH and MIMAULT (488) have stressed the connection between respiration and formation of soluble pectinic acids. MARTIN'S experiments (299) with apples showed that there are at least three kinds of processes which probably vary independently during maturation. Starch conversion, acid level and softening should be closely linked, but the change of colour has a rather independent character (see also p. 127).

In early investigations much attention was paid to the occurrence of the hypothetical enzyme protopectinase, which was thought to give rise to the formation of soluble pectic materials from insoluble protopectin. As discussed before, no reliable evidence has been produced that such a specific enzyme can be found in nature (see p. 74). Another possibility of formation of soluble pectinic acids depends on the occurrence of polygalacturonases, which have found to be present in several fruits. The solubility of pectic substances in the insoluble protopectin should be increased by a partial depolymerization as a result of attack on protopectin by these enzymes. A more severe depolymerization should account for the decrease of amount of pectic substances which is often found during maturation or senescence of fruits. However, no reliable evidence has been produced that polygalacturonases occur in apples (249, 259) and peaches (326), which has led to the development of other theories on formation of soluble pectinic acids.

HOBSON (218) found no polygalacturonase activity in fruits of *Vaccinium macrocarpum*, *Diospyros virginiana*, *Vitis vinifera*, *Citrus nobilis* (tangerine) and *Cucumis sativus*.

Finally, it has to be remembered that conversion of other cell wall constituents, which are intimately mixed with pectic substances, also takes place and may account for the loosening of pectinic acids from cell walls (see p. 14).

According to DAVIGNON (98), the decrease in firmness depends mainly on the formation of soluble polyosans, especially arabans, which are known to be associated with the pectic substances in the tissue.

Much attention has been paid also to the degree of esterification of pectic substances in fruits.

REEVE (394) investigated the degree of esterification of pectic substances in the tissues of ripening peaches with a histochemical method (alkaline hydroxylamine and Fe Cl_3). The degree of esterification was found to be 75–80% in immature fruits, whereas it increased just prior to ripening when it approached 100%. Then the degree of esterification decreased rapidly and the cell walls became apparently thinner as the ripening fruits softened. However, it has already been reported (see p. 54) that the results of these histochemical methods do not always correspond with the chemical estimation of degree of esterification of extracted pectinic acids.

STERLING and KALB (459) studied the degree of esterification of water-soluble and

acid-soluble pectinic acids from unripe to very ripe peaches and found a continuous decrease of degree of esterification from about 75% to about 50%. A rather striking decrease of degree of esterification during ripening of fruits has been reported also for pears (98, 326), tomatoes (522), cherries (98) and avocados (326); a slight or almost negligible decrease of degree of esterification of these pectinic acids is shown in many experiments on ripening apples (98, 131, 152, 522) and in Fig. 43.

During growth of Valencia oranges the degree of esterification of pectic substances in the peel increased rapidly to approximately 80% and remained relatively constant during the rest of the season (436).

Since the solubility of pectic substances is diminished by a partial saponification, it seems unlikely that pectinesterase activity is the real cause of the formation of soluble pectinic acids during ripening (459). However, a partial saponification of pectic substances may enhance the action of polygalacturonases (see p. 80).

DAVIGNON (98) found a maximum of pectinesterase activity in July-August in milled tissue of unripe Calville apples and in June in the tissue of Passe Crassane pears; in both fruits he noted a second and higher maximum at the end of the ripening stage and the beginning of senescence. WEURMAN (507, 509) has reported similar results for pears. POLLARD and KIESER (382) found considerable differences in pectinesterase contents of different apple varieties. According to DAVIGNON (98), the maximum pectinesterase activity in cherries occurs in the ripe fruits. In ripening tomatoes HOBSON (219) showed that the pectinesterase activity was increased when the fruits became fully red, whereas in 'blotchy ripening' tomatoes the activity was lower than in normal ripening fruits.

HOBSON (220) proved that polygalacturonase and pectinesterase activity in tomatoes was stimulated by nitrogenous fertilizers, whereas potassium increased the activity of pectinesterase.

Attempts have been made to explain changes of pectic substances in fruits from variations of the enzyme activities found in the disintegrated tissue.

MCCREADY and MCCOMB (326) studied the polygalacturonase activity of peaches, pears and avocados. No pectin degrading polygalacturonases could be demonstrated in the unripe fruits, but in ripe pears and avocados the activity of such enzymes could be shown. However, no evidence was produced for the presence of this enzyme in ripe peaches. WEURMAN (507, 508, 510) investigated also the polygalacturonase activity of pears. He reported that, in some varieties of pears, polygalacturonase inhibitors are found which may mask the presence of such enzymes. The same may be expected in other fruits. DAME ET AL (94) showed that intrinsic viscosity and equivalent weight of the versene-extracted pectic substances of Bartlett pears decrease during ripening, indicating glycosidic hydrolysis by polygalacturonase and deesterification by pectinesterase. A similar drop of intrinsic viscosity of versene-extracted pectinic acids in ripening peaches was found by POSTLMAYR ET AL (387).

Though the solubilization of pectic substances in many fruits may be explained by the activity of polygalacturonases no reliable evidence of the occurrence of such enzymes in apples has been produced (249, 259). KERTESZ (258) and GRIFFIN and KERTESZ (183) have suggested that it should be possible for solubilization to be effected by the action of ascorbic acid or hydrogen peroxide on plant tissues (see p. 51), which may cause depolymerization of pectic substances.

Since most theories on solubilization of pectinic acids take into account a partial depolymerization of pectic substances, DOESBURG (131) has paid special attention to this point in relation to apples and proved that there is no evidence of partial depolymerization of pectic substances in these fruits during ripening. This conclusion was based upon the constant jellying power of pure acid-extracted pectins produced from maturing fruits. DOESBURG (131) found no difference of jellying power of

acid-extracted-insoluble pectins and the naturally occurring soluble pectins after the same acid heat treatment. This is shown in Table 8.

TABLE 8. *Characteristics of pectin fractions from apples (harvest 1951) after heating 2 hrs. at 85° C (tested after storage of apples at 10° C in Febr. 1952).*

Variety	Pectin fraction in apples	Percentage of total pectin in apples	Jellying grades of pure pectins	Degree of esterification
Belle de Boskoop	water soluble	37.1	383	74.2
	oxalate soluble	13.1	377	73.1
	soluble after acid extraction	49.8	387	74.1
Cox's Orange Pippin	water soluble	37.9	377	74.3
	oxalate soluble	15.3	374	74.3
	soluble after acid extraction	46.8	371	74.9

The foregoing results indicate that no decomposition of polygalacturonic chains occurs during pectin solubilization in apples (see Fig. 32).

EGGENBERGER (152) also tested the jellying power of pectins extracted from apples during development and storage and found a maximum jellying power at the time of harvesting. However, this results may have been influenced by slight changes in the degree of esterification which influenced the pre-setting of jellies and the relation between jellying power and pH of test jellies (see p. 39). When these phenomena are taken into account the maximum jellying power at the time of harvesting can be explained because the results of jelly tests, as performed by EGGENBERGER, do not depend on the degree of polymerization alone. EGGENBERGER did not find a distinct relationship between results of viscosity measurements and jellying power of extracted pectins. From his results it is clear, however, that soluble pectins in apples are not degraded.

Because no breakdown of pectic substances could be found in maturing apples and from several other indications DOESBURG (131) supposed that during ripening a movement of calcium in the cell walls takes place, caused by an increased intake of calcium by the living cells, or by the formation of calcium-binding compounds in the cell walls. In both cases it is to be expected, on grounds of electrostatic neutrality, that there will be an exchange of cations (particularly potassium and hydrogen ions) from the contents of the apple cells with the calcium bound by absorption, or through salt formation by the pectic substances in the cell walls. As a secondary effect of this cationic exchange, a lowering of pH of cell walls during pectin solubilization would be expected. Indeed, several indications have been found that a calcium movement and decrease of pH of cell walls takes place during pectin solubilization. These changes were found to be related to changes in the amount and constituents of the mixture of organic acids in the fruits (131).

Removal of calcium causes a swelling of the insoluble protopectin and may promote the solubilization of pectin. STERLING and KALB (459) have pointed out that this effect will be counterbalanced by the firming influence of a simultaneous decrease of pH of cell walls. However, as shown in Figs. 7 and 12, removal of calcium may have a greater weakening effect than the firming result of a decrease of pH.

Changes in water-soluble calcium and magnesium content of pear fruit tissue during maturation and ripening have been studied in relation to changes in pectic substances by ESAU, JOSLYN and CLAYPOOL (158). Their data indicate that the total pectin, the water soluble pectin, total calcium and total magnesium are correlated with firmness and that the relationship of soluble calcium and magnesium to firmness is radically different in detached fruit from that in fruit on the tree. According to their results the 'bound' calcium and magnesium are present in concentrations far greater than the total available carboxyl groups of pectic substances and protopectin content is not related to calcium- or magnesiumbound pectinic acid chains.

In the discussion on protopectin structure (see p. 16) it was stressed that it is not likely that high-methoxyl pectins, which can be extracted from protopectin in apples, are rendered insoluble by calcium salts, but that instead the calcium simply decreases the swelling. For this reason it is to be expected that other factors must be involved in solubilization of pectins when no polygalacturonase activity is found. Such a factor may lie in the conversion of other cell wall constituents which are closely associated with pectic substances in cell walls.

JERMYN and ISHERWOOD (235) suggested that after a certain point of natural growth before harvesting, the cell wall is largely static in nature. They supposed that this may result from a balance between the rate of breakdown of carbon compounds due to respiration and the supply of nutrients from the tree. After picking, this balance is disturbed. The fall in the total amount of polysaccharides found in pears, detached from the tree, is interpreted to mean that, at least a part of the carbon compounds in the cell wall become available for conversion to carbon dioxide. Araban was found to be the most labile material in ripening Conference pears (235). The cell wall appears to be in dynamic equilibrium with the cytoplasm and the apparently static nature of the cell wall in the fruit on the tree will not reflect its full relationship with cytoplasm. HALLER (188) and ESAU ET AL (158) also reported a different behaviour of pectic substances in ripening fruits according to whether they were detached from the tree or not.

DAVIGNON (98) has pointed out that the rather rapid decrease of degree of esterification, during maturation and senescence of pears and cherries, is connected with a decrease of pectic substances during these periods, whereas the nearly constant degree of esterification of pectic substances in apples is related to a reasonably stable amount of pectic materials. According to DAVIGNON, these facts suggest that the polygalacturonase activity in fruits may be dependent on their pectinesterase activity (see p. 80). However, it must be stressed again that there is no real proof of the occurrence of polygalacturonases in apples and that no indication of depolymerization has been found during the period of partial solubilization of pectic substances.

DAVIGNON (98) reported that the estimation of polygalacturonase activity in several species of fruits is rather difficult. He found that, during growth of fruits, oligo-uronides were converted into pectic substances. However, during maturation, the amount of oligo-uronides increased to a constant value when the amount of pectic substances was also constant (apples) or, increased further when pectic substances are broken down during this period (pears). For this reason, DAVIGNON supposed that, during the early stages of maturation, oligo-uronides are formed by the fruits, but in this period their ability to form pectic substances is absent. The increase of oligo-uronides during senescence should result from a breakdown of pectic materials.

FODA (164) studied the relation between firmness, pectic substances and pectolytic activity in tomatoes. The inner wall tissue of these fruits, ripened at 15° C, softened more readily than at 30° C, whereas protopectin decreased and water soluble pectinic acids accumulated more rapidly at 15° C. The low-methoxyl pectinic acid fraction (NH_4-oxalate fraction) was rather high at 30° C, which he supposed to be due to the more favourable temperature for the pectinesterase. Pectolytic activity increased very rapidly from three to six days after turning, and reached a peak at twelve to fifteen days; the rapid increase coincided with the period of the major decrease in firmness.

By the use of paper chromatography, ASH and REYNOLDS (12) detected free galacturonic acid in several varieties of pears after ripening at 20° C, but not in green or tree-ripened pears. Similar results were obtained with freestone peaches, but no free uronic acid was found in apricots whether ripened on the tree or at 20° C. MCCLENDON, WOODMANSEE and SOMERS (308) found a very low amount of free uronic acids in unripe tomatoes and in unripe and ripe apples. In ripe tomatoes and overripe apples, a ten fold increase of these compounds could be shown to be present.

Influence of ionizing radiations on pectic substances in fruits and vegetables

As reported by GLEGG and KERTESZ (180) and KERTESZ ET AL (264), cellulose and pectic substances are degraded by ionizing radiations without any significant change in degree of esterification of pectinic acids. The breakdown of pectin in solutions by irradiation with γ-rays is reduced by the presence of sugars; the degradation being

shown to be completely eliminated when radiation was applied to jellied mixtures (e.g. with 60% fructose and 0.35% tartaric acid).

The application of ionizing radiations to fruits and vegetables causes softening of tissues (59, 81, 181, 266, 306) accompanied by a decrease of protopectin content and an increase of soluble pectic substances or with some decrease of the total amount of pectic substances (306, 449).

According to GLEGG ET AL (181), different plant tissues required a variable minimum dose to cause softening. When using γ-rays, this minimum dose was 34.7 Krad for Gravestein apples, 166 Krad for Chantenay carrots, and 316 Krad for Detroit dark red beets. MCARDLE and NEHEMIAS (306) postulated that the lower sensitivity of carrots (as compared with apples) should be due to the presence of supporting of fibres of cellulose and hemicelluloses. As shown by KERTESZ ET AL (266) in apple tissues and carrots, the degradation of cellulose and pectic substances occurred at approximately the dose at which tissue softening could first be demonstrated and progressed with increasing dose. In beets, the softening of tissues did not seem to be as clearly related to such marked changes in pectic substances and cellulose.

CLARKE (81) studied the changes of texture and pectic substances of Cox's Orange Pippin apples after irradiation and during storage at 3 °C. The irradiated samples (doses 100–200 Krad) showed some softening and formation of soluble pectinic acids immediately after the radiation treatment but, during subsequent storage, these phenomena took place at a more rapid rate in untreated fruits. After the storage period, no significant differences could be found between treated and untreated lots.

SOMOGYI and ROMANI (449) pointed out that pears, peaches and nectarines irradiated (300–600 Krad) under nitrogen atmosphere softened much less than fruits irradiated under air. Perhaps this difference may be due to reduced formation of free radicals under anaerobic conditions. However, this hypothesis was not confirmed by exposure of pectin solutions to γ-rays under similar atmospheric conditions.

SOMOGYI and ROMANI (449) also found an immediate softening effect of ionizing radiations (300, 600 and 900 Krad) on fruits. After four days, the control peaches became so soft by normal ripening that no differences could be found between the control and fruits irradiated with different doses. In some cases, pears showed recovery of firmness as well as a delay in the rate of softening from normal ripening. It has been shown many times that irradiation of fruits may cause a delay of ripening and that the metabolism of fruits (respiration) is affected also by low-dose irradiation which may also influence the activity of fruit enzymes. SOMOGYI and ROMANI (449) showed that application of lower radiation doses (200 and 500 Krad) to cherries resulted in a higher pectinesterase activity, when extracted immediately after irradiation. After 4 days the same pectinesterase activity was found in irradiated and untreated fruits.

Chapter 6

Pectic substances in preserved horticultural products

Influence of preservation on texture and firmness

Edible tissue of fruits and vegetables consists largely of parenchyma tissue with minor amounts of anastomosing xylem and phloem elements.

This parenchyma tissue is mostly composed of thin-walled isodiametric or cylindrical cells with a living protoplast. The cells may be storage cells, containing a greater amount of starches and sugars (in roots, tubers, seeds and fruits) or synthesizing cells containing the green chlorophyll (leaves and stems, unripe fruits). Mostly the cells of parenchyma tissues do not fit together perfectly, thus forming intercellular spaces, filled with air.

The relation between properties of various plant tissues and their edible quality has been discussed by WEIER and STOCKING (505). In mature plants, other tissues may cause woodiness, stringiness or corkiness. In such cases, these tissues have to be removed.

The conduction tissues can be divided in two groups: the water conducting xylem and the food conducting phloem. The walls of xylem cells are lignified, so that the presence of large amounts of xylem may be objectionable.

The same effect may be caused by the presence of greater amounts of supporting tissues, whether composed of fibers or collenchyma cells. This is especially true of fibers which have become lignified.

Vegetables and fruits are often protected by an epidermal layer, which may be regarded as specialized parenchyma cells; the outside of the epidermis is covered with a thin cutin layer. Frequently hair cells form an important cell type in the epidermis.

In roots, the walls of cells under the epidermis are usually impregnated with suberin, thus forming a corky layer. Corked cell layers are seldom found in leaves, but may be present on the outside of fruits and tubers (potatoes).

The following discussion will be restricted to the behaviour of pectic substances in parenchyma tissues, when they are cooked, crushed or preserved, and to the relation between the behaviour of pectic substances and the structure of edible plant products.

Since the pectic substances are regarded as the cementing materials between plant cells the firmness of non-disintegrated plant tissues is considered to be greatly dependent on the properties of these substances in the tissues. It has to be kept in mind, however, that several other factors may exert their influence upon the firmness of plant tissues of parenchymous nature which need to be mentioned here but briefly.

During cooking and all modes of preservation, the cells are killed and the semipermeability of the cytoplasm will be destroyed. When the water-binding capacity of the contents of the cells is low, it is to be expected that cell-death will result in a loss of solutes and water. For this reason the crisp firm texture of most fresh plant tissues, which is chiefly due to cell turgidity, is lost.

In living turgescent tissues, the vacuole in the cells becomes large, pressing the water-rich protoplasm against the partially elastic cell wall, which is thus stretched. Cell-death results in an increase of permeability of the protoplasm, thus enabling the diffusion of solutes out of the cells to regions of lower concentration. This loss of solutes will be accompanied by a loss of water. The cell walls are no longer stretched by the osmotic pressure in the vacuoles; by contraction of cell walls, cell juice also is pressed into the intercellular spaces.

However, not in all cases will the loss of turgidity be complete. The loss of water will be limited when a relatively high amount of hydrophilic colloids is present in the cells. It has to be remembered that, during heating, the starch will be gelatinized, which causes an increase in possible points of hydration and an increased swelling capacity. In heat-treated pulses and potatoes, the gelatinized and swollen starch may fill the cells completely and stretch the cell walls again, in some cases breaking through the walls.

When a great loss of water and solutes occurs, the flabbiness of the tissue will be apparent and the remaining firmness of tissues will depend mainly on the rigidity of cell walls and the strength of contacts between the cells, both of which are partially influenced by the behaviour of pectic substances.

The firmness of tissues, relatively poor in hydrophilic colloids, is also influenced by the dimensions of the cells. A tissue with large cells may show a greater flabbiness than a tissue with small cells, which contains higher contents of cytoplasmic and cell wall materials. Products, not grown under optimal conditions, often contain smaller cells and possess a greater firmness. REEVE and NEUFELD (397), CARTER ET AL (71) and PROEBSTING ET AL (389) have studied the influence of nitrogen nutrition of freestone peaches upon their processing quality. REEVE and NEUFELD (397) found that cellular distinctions between high- and low-nitrogen peaches could serve to explain differences in texture and appearance of the canned fruits. The high-nitrogen freestone peaches have much smaller cells than low-nitrogen fruits. After canning the high-nitrogen fruit remained firm and finely textured while the low-nitrogen product was coarse, stringy and often ragged in appearance.

Finally it has to be mentioned that freezing may exert a considerable influence on texture of products which is not related to changes in chemical composition of pectic substances. The influence of freezing may be summarized in the following points, mentioned by WOODROOF and cited by TRESSLER and EVERS (479): 1) ice crystals may puncture the cell walls or pierce between the cells; 2) flabbiness may be due to withdrawal of more water from cells into intercellular spaces during freezing than is reabsorbed on thawing. In the case of products containing gelatinized starch, no leakage and very little loss of structure is apparent, which is due to the capacity of such materials to reabsorb large quantities of water; 3) freezing destroys the colloidal complex of cells and reduces turgidity. The colloidal state of the cytoplasm of frozen and thawed mature fruit tissue is so completely destroyed that it is doubtful if complete active absorption may occur.

The influence of the rate of freezing upon texture is well known, especially for non-starchy products. With more rapid freezing, the size of ice crystals is much smaller and the number of ruptured cells is reduced.

Preservation of fruits and fruit pulps with sulphur dioxide and by freezing

When fruit tissues have been killed and are preserved by addition of sulphur dioxide or freezing, enzymic degradation of pectic substances may take place, whenever pectolytic enzymes are not inactivated or inhibited.

As has been mentioned before some fruits contain depolymerizing enzymes and all fruits may be expected to contain pectin methylesterase. According to CHARLEY (74), most of the polygalacturonase action found in soft fruits is due to microbiological action on fresh fruits.

WHITE and FABIAN (512) cultured specimens of six genera of moulds most commonly found on black raspberries in pectin containing media. All decreased the viscosity of a citrus pectin medium and four that of an apple pectin medium. On a medium containing extract of black raspberries, *Pullularia* sp., *Fusarium* sp. and *Penicillium* sp. exhibited pectolytic activity; *Alternaria humicola, Cladosporium* sp. and *Botrytis cinerea* did not.

The fungal contamination of Kentish strawberry fruits was investigated by LOWINGS (288); *Sphaerotheca humuli, Botrytis cinerea* and *Mucor piriformis* were found to produce $\pm$ 98 % of the mycelium present on fresh and stored strawberries. A minor amount of mycelium was produced by *Cladosporium herbarum, Penicillium* sp. and *Trichoderma viride. Klockeria apiculata* was found to be the most frequently occurring yeast; it caused a slow breakdown of fruits when inoculated on bruised strawberries.

The incidence and pectolytic activity of fungi isolated from Michigan strawberry fruits were studied by BENEKE, WHITE and FABIAN (42). Some of the samples of fruits were surface treated by immersion for 1 minute in a solution containing two parts of sodium hypochlorite and one part of 70 per cent ethanol. It was shown that moulds could be isolated from the subsurface of green as well as ripe fruits; when comparing the number of isolations from the subsurface of green fruits and ripe fruits a marked increase during ripening could be found. When the various genera of fungi were inoculated into fresh strawberry fruits, strains of *Botrytis cinerea, Rhizopus nigrificans* and *Aspergillus* sp. were shown to produce macroscopically greater degradation than *Pullularia pullulans, Trichoderma* sp., *Penicillium* sp., *Hormodendron* sp. and *Alternaria* sp.

Similar results of the presence of moulds and yeasts in strawberry fruits with no macroscopically visible mould growth have been reported by STADEN and DOESBURG (453). It appeared again that a more or less specific microflora can be found in immature strawberries. In this flora, organisms like *Pullularia pullulans*, which produce pectolytic enzymes, are present.

Since the pectolytic activity of different moulds is not the same, the pectolytic activity produced by moulds cannot be estimated by application of the Howard-test.

It is an essential feature of a pulping or any other preserving method of fruits that it should prevent the enzymic breakdown of pectic substances as far as possible. According to MARTIN (300), application of heat is the best way to destroy enzyme activity, e.g. heating for 10 minutes at 85 °C or two minutes at 90 °C, but inhibition of pectic enzymes is attained also by freezing.

In products which are rich in ascorbic acid, some oxidative degradation of pectic substances may be expected; this effect is checked by freezing or addition of SO_2. In frozen or sulphited pulps, this mode of destruction does not occur, (see p. 51) but it may be expected in fruit pulps preserved with non-reducing preservatives, e.g. benzoates (226, 300).

When pectic enzymes are inhibited or inactivated and the oxidative processes are also inhibited, some changes in pectic substances may occur as a result of the influence of pH and temperature during storage of pulp. An example of the influence of

these factors on pectic substances is shown in Fig. 25. Further the properties of cell walls may be altered and may cause disintegration of fruits also.

In the next section the effect of enzymic activity will be discussed mainly in relation to the behaviour of fruit pulps to be used for jam manufacture in Europe. Thereafter some attention has to be paid to non-enzymic changes caused by pH and temperature.

The influence of pectic enzymes in various fruits preserved with sulphur dioxide may vary according to the respective activity of various types of pectic enzymes. Experiments on the behaviour of fruit pulps, preserved by freezing or addition of sulphur dioxide, have been made by MEHLITZ (333, 334), MORRIS (340), HOTTENROTH (226) and DOESBURG (127). In pulps of several unheated soft fruits, preserved with SO_2, the activity of depolymerizing enzymes may be serious, causing a breakdown of pectic substances, e.g. in raspberries, black and red currants and gooseberries (127, 226).

This breakdown of pectic substances causes a rapid loss of jellying power. Moreover disintegration of fruits mostly occurs when the cementing pectic substances between the cells were decomposed.

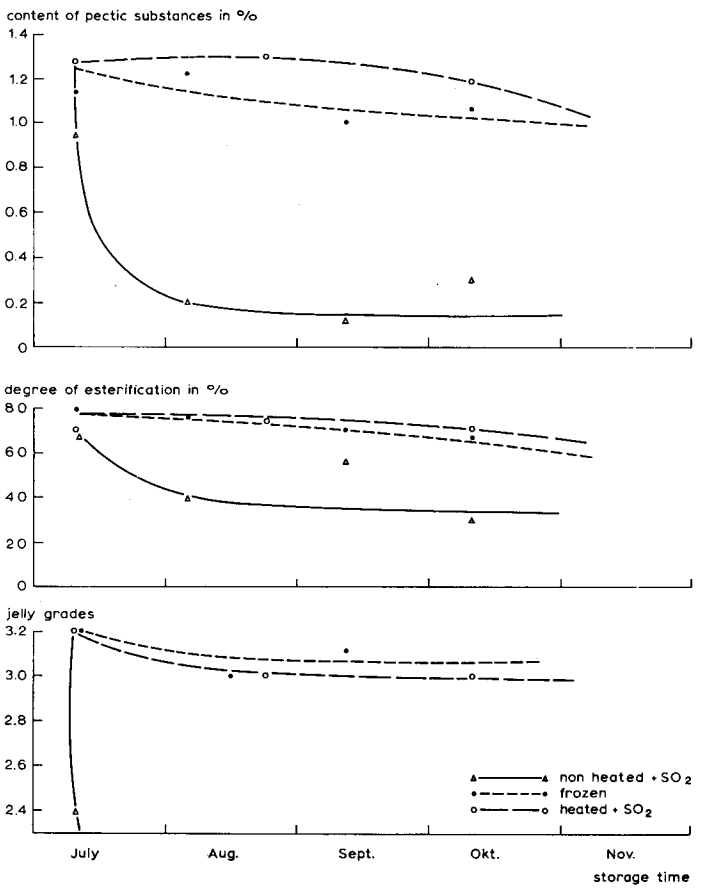


FIG. 46.
Behaviour of pectic substances in black currants during freezing and storage at —18°C, and during preservation (with and without previous heating) with SO_2 (Doesburg, 126).

The behaviour of pectic substances in black currants during freezing and storage at —18 °C and during preservation with sulphur dioxide (previously heated or not heated) is shown in Fig. 46. As it might be expected, the amount, degree of esterification and jellying power (calculated on fresh fruits) of pectic substances are reasonably stable in frozen pulp or in pulp previously heated before preservation with SO_2. Rather greater changes occur in non-heated pulp preserved with SO_2. Similar results have been obtained with most soft fruits.

However, not all fruits undergo such serious breakdown of pectic substances when they are preserved with SO_2 without a heat treatment, e.g. the breakdown is usually quite slight in pulps of strawberries, cherries, plums and apples.

When the fruits contain pectic substances of a more than negligible jellying power it is ensured that loss of this jellying power is prevented by a heat treatment before addition of SO_2. It must be remembered that even a very low activity of depolymerizing enzymes causes a heavy loss of jellying power (Fig. 31). For these reasons apples, plums, red and black currants and gooseberries have to be heated (121) when they are preserved with SO_2. Especially, in the case of apples, it is also important that the pectic substances are solubilized when the fruits are steamed and milled, since complete solubility is necessary to obtain its maximum jellying power.

The jellying power of pectic substances from raspberries, cherries and strawberries is very low. However, it is customary to produce jams of strawberries and cherries which contain non-disintegrated parts of fruits. For this reason the influence of pulping upon the texture of these fruits is very important.

Strawberries, which are pulped with SO_2, are not heated before pulping, because such a heat treatment may cause a considerable loss of soluble solids, flavour and firmness. Since it is known that the addition of calcium salts has a firming action on strawberry fruits (see p. 112) a part of the added SO_2 is replaced by a solution of calcium bisulphite.

For the manufacture of strawberry pulp, preserved with SO_2, the fruits are picked without calyx, washed and filled into barrels; 12.5 l water and 5 l SO_2-solution (5 %) are added to 135 kg strawberries.

From experiments by GERSONS and KARELSE (179), it could be concluded that the appearance of jams made from strawberry pulp, was very poor when less than 40 % of the total amount of SO_2 had been replaced bij SO_2 from calcium bisulphite, because all fruits in the jam were disintegrated. Higher percentages of Ca-bisulphite influenced the outer appearance favourably, showing the strawberries as separate fruits in the finished jams. Replacing more than 80 % of the total SO_2 by calcium bisulphite resulted in non-disintegrated fruits, that were however too tough. In general, it could be concluded that use of 50–60 % of total SO_2 in the form of calcium bisulphite was optimal. However, in later experiments (176) it was shown that the optimal amount of calcium is dependent upon several unknown factors and may correspond with an amount of calcium bisulphite varying from 30–70 % of the total amount of SO_2.

In most cases a reasonable quality of strawberry pulp can be obtained by the formulas mentioned above, but in some cases after some months the fruits show a severe decrease of firmness or even a complete disintegration as shown in Fig. 47. This behaviour is related to the presence of a greater activity of depolymerizing pectic enzymes.

In some cases severe softening or disintegration has been reported also in raw

FIG. 47.
Sulphited strawberries of good quality or showing complete disintegration.

brined cherries (62, 282, 455, 523) from which maraschino type cherries are made. The brine is made up of 1.25% sulphur dioxide and 0.75% calcium hydroxide, which is acting as a firming agent. As shown by LEWIS, PIERSON and POWERS (282) the softening of raw brined cherries could be caused by addition of cherries infected with *Aspergillus niger*, *Cytospora leucostoma* and *Penicillium expansum*, but was not initiated by addition of cherries rotted by *Alternaria* sp., *Aspergillus oryzae*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium* sp., *Mucor racemosus*, *Rhizopus stolinifer* and *Sclerotinia fructicola*. However, the breakdown in commercial products is of sporadic occurrence and for this reason it is believed that infection with *A. niger* and *P. expansum* are not the usual cause of this severe softening, since these moulds produce about the same amount of rotted cherries every year. For this and other reasons it is probable that the sporadic occurring severe breakdown is correlated with the presence of *C. leucostoma*, which also causes die-back of cherry trees.

YANG, STEELE and GRAHAM (523) and BREKKE and WATTERS (62) showed that the depolymerizing enzyme in brined cherries can be inactivated by the addition of 0.01–0.025% commercial alkyl aryl sulphonate to the brine. By use of radioactive isotope tracer techniques, 0.6 ppm of residual alkyl aryl sulphonate was found to remain in the leached cherries when 0.01% of a commercial preparation of alkyl aryl sulphonate was added to the original brine. No undesirable effects were observed on maraschino cherries when made from brined cherries treated with as much as 0.1% of commercial alkyl aryl sulphonate.

Several attempts have been made also to detect the cause of the disintegration of fruits in raw brined strawberries (177, 370, 453, 454), which has been mentioned above.

According to PANDHI (370) it is of interest that, in his experiments with quite fresh strawberries preserved in sulphur dioxide solution in clean glass jars, these fruits never showed any signs of breakdown as long as sufficient sulphur dioxide concentration was maintained to inhibit mould growth. On the other hand it has been found that strawberries allowed to stand at room temperature for 24 hours before being put into preservative showed some breakdown in a few months. To prevent breakdown, PANDHI makes the following recommendations: 1) only sound whole strawberries should be used for preservation; 2) strawberries should be put down in sulphur dioxide solution as soon as possible after picking; 3) baskets should be waxed, if possible metal trays should be used instead of chip baskets; 4) barrels should be cleaned thoroughly and preferably steamed as soon as they are emptied; 5) mould growth on strawberries may often be considerably reduced by rapidly cooling after picking.

However, to the experience of the author the disintegration of pulped strawberries is not really reduced when using these recommandations. Certainly a hygienic treatment of fruits to prevent mould growth is preferable for several reasons, but in contrast with the results of PANDHI, strawberries showed disintegration even when they had been preserved immediately after picking; this deterioration is not decreased by washing, even when detergents are used by which the mould counts are decreased to 10 % of the original value. Further a benificial effect of cooling could not be observed. These facts strongly indicate that contamination with moulds after picking is not the most important factor in relation to the quality of the preserved fruits and that, in most cases, the pectolytic enzymes are present in the fruits when they are harvested. This may be understood when the data on mould growth in green and ripe strawberries, which have been mentioned before, are taken into account.

It has often been observed that the disintegration or softening of strawberries is related to weather conditions before harvest. After a dry growing season, the number of samples which show this kind of spoilage is very low; it is increased when fruits are harvested after a wet growing season or when heavy rainfall has occurred some days before harvest (177). No relation could be observed between behaviour of strawberries in the pulp and the type of soil on which they had been grown or with the manuring treatment.

Nevertheless, GERSONS, DOESBURG and SCHURINK (177, 528a) reported that some relationship exists between the source and the behaviour of pulped strawberries, since it could be shown that within the strawberry producing area in the southern part of the Netherlands distinct differences between the quality of pulped fruits from ten different neighbouring regions (municipalities) could be found. This effect was shown in the 1952 and 1953 seasons, when the spoilage was occurring rather frequently; in both years from every region 30–50 samples of the Jucunda variety, grown on different plots, have been preserved and their behaviour during storage studied.

In 1953, soil samples were taken from similar plots in the ten different regions; from every plot some soil was added separately to aliquot parts of a lot of strawberries, which had been previously heated during 10 min. at 90° C; then every aliquot, containing an amount of one soil sample, was also preserved with sulphur dioxide solution. In a number of these aliquots disintegration of strawberries also occurred during storage. In Fig. 48 the relation is shown between the frequency of disintegration in raw strawberry pulp samples from different regions (arranged in order of increasing number of disintegrated samples) and the frequency of this spoilage when soil samples from these different regions had been added to heated strawberries.

From these results it may be concluded that there is a rather strong relation between the influence of soil samples and the behaviour of preserved raw strawberries which are produced from these soils.

Since no growth of micro-organisms could be found in the samples, even when soil had been added, it is evident that different soil samples contained a different amount or type of pectolytic enzymes, which has to be related to a different microbiological flora in these soils. Dependent on weather and other growing conditions it may be expected that the fruits will be attacked by several types of micro-organisms and that disintegration or severe softening of preserved fruits will occur most frequently when the strawberries have grown in the presence of a microbial flora, by which suitable pectolytic enzymes are produced. Up till now no indications have been found of which organisms are the most important in relation to disintegration of strawberries.

Certainly other factors have to be taken into account also. It has been shown that there are differences between different varieties. Further, the incidence of disintegration

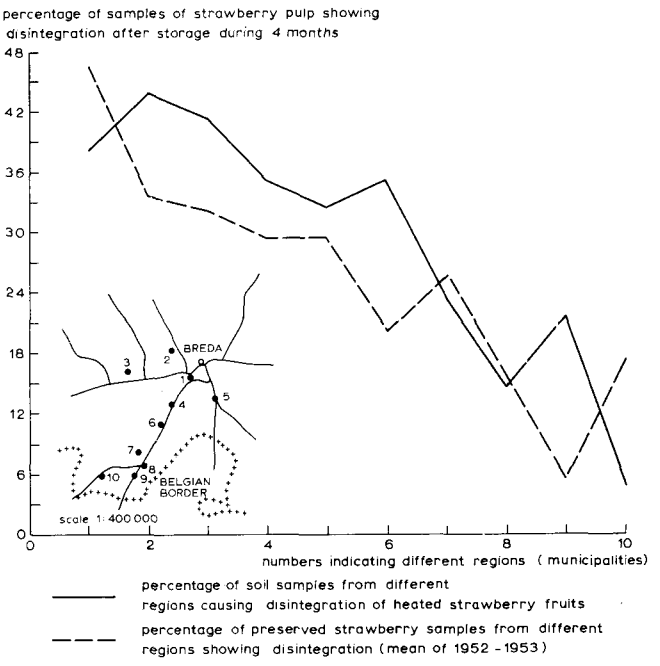


FIG. 48.
Relation between percentage of disintegrated sulphited strawberries of total number of samples from different municipalities and the percentage of soil samples from these municipalities which induced disintegration in heated sulphited strawberries (Gersons, Doesburg and Schurink, 177).

may be related to the health of the crop which may influence the possibility of infection by moulds during development of fruits. Various factors have been discussed also by SISTRUNK ET AL (438) in relation to the breakdown of frozen sliced strawberries.

In the foregoing discussion, attention has been paid to the influence of depolymerizing enzymes which may destroy the jellying power or the texture of pulped fruits. In some cases, however, the action of pectinesterases is also important.

When no breakdown of pectic substances occurs, the action of pectinesterases in sulphited strawberries is often apparent. In these cases the pectinic acids in the juice from the fruits are converted into pectic acids and low-methoxyl pectinic acids which form a jellied mass, since they are made insoluble by the low pH and the calcium content of the product. The presence of such a jelly is always connected with the presence of firm, non-disintegrated fruits. The occurrence of jellying indicates that no depolymerization of polygalacturonic chains has taken place. Moreover, the fruits are stiffened by the jelly formation.

Change in texture of frozen Montmorency cherries may be caused also by pectinesterase activity, as shown by GEE and MCCREADY (173). Frozen cherries toughened when they were stored at temperatures of 20°F or higher, but were stable at −10°F. This toughening of texture was noticeable after two weeks and progressively increased with longer periods of time. It could be attributed to an enzymic deesterification and the formation of a calcium pectinate gel from calcium present in the fruit tissue.

Toughening of cherries has been reported many times when raw fruits are preserved with sulphur dioxide. It is probable that this effect is also caused by pectinesterase activity; it can be prevented by steaming of fruits previously to preservation.

As shown in Fig. 33 a slight pectinesterase activity occurred also in frozen plums and gooseberries during storage at —18 °C. As has been reported by PILNIK (376), in frozen orange concentrates pectinesterase activity is noticeable when the product is stored at temperatures somewhat higher than —18 °C.

In the foregoing discussion, attention has been paid mainly to enzymic action in fruits preserved with sulphur dioxide or frozen. However, the relatively low pH of fruit pulps may exert also some influence on pectic substances and texture of fruits by decomposition of cell wall constituents.

In frozen fruits the influence of pH on pectins will be negligible; in these products the influence of the preservation method on texture of fruits will depend mainly on the rate of freezing, addition of sugar, etc. as has been described by SISTRUNK ET AL (438) for frozen sliced strawberries.

MORRIS (340) and HOTTENROTH (226) showed that in several instances the jellying power of heated fruit pulps, preserved with SO_2, increased during storage. According to HOTTENROTH (226) this effect may be attributed to partial deesterification of pectins and to the formation of soluble pectins from insoluble protopectin. However, in the opinion of the author, the influence of partial deesterification may be neglected, since this effect has been shown to be very slight when pectins are stored in solutions with a pH $\pm$ 2.5–$\pm$ 3.2 which corresponds with the fruits pulps (see Fig. 25).

According to SABOUROFF and KALEBIN (415) the decrease of degree of esterification in stored sulphited apple pulps is very small indeed. They showed that some solubilization of pectin takes place during storage of pulp of raw preserved apples, but these pectins are solubilized also when the pulp is heated before preservation with SO_2 or when it is used in jam manufacture. According to the author, the increase of jellying power of pectins during storage of pulps of plums or some other fruits may be due to the removal of side groups attached to the polygalacturonan chains. In relation to this, it has to be mentioned that WADE (500) found a decrease of acetyl contents of total cell wall polysaccharides during storage of canned or sulphited strawberries. He noted also a progressive decrease of insoluble polygalacturonic substances during storage of canned or sulphited strawberries; similar changes even occurred in the frozen fruits stored at —18 °C.

When solubilization of pectic substances takes place during storage of fruit pulp preserved in sulphur dioxide as is to be expected, the texture of fruits is also affected. This effect has been demonstrated by STADEN and DOESBURG (454) when studying the influence of pH on the disintegration of raw pulped strawberries. An attempt was made to reduce the activity of pectolytic enzymes by decrease of the pH of strawberry pulp, since the optimum pH of pectolytic enzymes is higher than the pH-value of commercial pulp (pH $\pm$ 3).

In order to obtain more information on the influence of the pH-value on the behaviour of strawberry pulp, strawberries from different sources were processed into pulp samples with pH-values ranging from $\pm$ 1.5 tot $\pm$ 4.0. As could be expected the disintegration was greatly intensified by a rise in pH-value, when this then approaches the pH-optimum of pectolytic enzymes. On the other hand, the fruits appeared to weaken considerably or to disintegrate when the pH-value was reduced to $\pm$ 2.0 or $\pm$ 1.5; this effect cannot be attributed to enzymic activity, but is caused by a chemical decomposition of cell wall constituents (protopectin or hydrolysis of hemicelluloses). A pH-value of $\pm$ 2.5 to $\pm$ 3.0 appeared to be optimal for the retention of original texture (454).

Pickling of products, sauerkraut

Products which are preserved by pickling or similar preservation methods may show softening of tissues as a result of enzymic action as has been described in fruit pulps. Since by these manners of preservation anaerobic conditions are prevailing no attention has to be paid to oxidative breakdown of pectic substances.

As shown by DAKIN (93) oxidative deterioration can be found when shredded red cabbage is acidified with acetic acid and placed in open containers.

This softening of tissue could be prevented by addition of SO_2 (100 ppm), iodine, sequestering agents, ethanol, formaldehyde or by placing the product into a closed container. As postulated by DAKIN this softening should not be caused by microbial action, nor by oxidation of ascorbic acid (see p. 51). DOESBURG (129) showed that ascorbic acid is very stable in shredded cabbage under aerobic conditions. Formaldehyde is known to exert a firming action on plant tissues.

Analysis of pectic substances showed that, during softening, a part of the insoluble pectic substances are solubilized and the total amount of pectic substances remained unaltered. This solubilization may be due to partial depolymerization without distinct decrease of the amount of pectic substances.

It is known that a rather strong partial depolymerization may take place without a detectable loss of pectic substances (see Fig. 31). KERTESZ (258) and GRIFFIN and KERTESZ (183) have postulated that on plant tissues similar effects can be affected by the action of ascorbic acid alone or in combination with hydrogen peroxide. However, as mentioned before, according to DAKIN (93) another non-enzymic factor occurs in red cabbage, capable of directly or indirectly carrying out an oxidative degradation of cellulose under acid conditions. The change of protopectin to soluble pectin, which apparently accompanies the loss of cellulose from the tissue, can be explained either on the assumption of a similar oxidative factor affecting protopectin, or alternatively that the insolubility of the protopectin is derived from its association with cellulose.

Much attention has been paid to the deterioration of fruits in cucumber salt stock (35, 37, 40, 102, 159, 161, 189, 239, 275) since cucumber softening during curing resulted in large losses to the pickle industry. This softening of tissue is shown to be due to the breakdown of pectic materials by pectolytic enzymes (35).

The main purpose of brining is to preserve the cucumbers until the packer is ready to manufacture them into various types of pickle products. This is a necessity, since the cucumber growing season lasts only a few weeks, but the packing plants are operating throughout the year. There are many different salting procedures.

According to DEMAIN and PHAFF (102) most of the current procedures in U.S.A. are based on the same principle. The fresh cucumbers are placed in large wooden vats containing brine and covered with wooden boards to prevent them from floating above the brine surface. Brine of sufficient strength is added to give a salt concentration of 8 to 10%, after equilibrium between cucumbers and brine. Periodically the salinity is determined and increased by addition of dry salt, according to the individual procedure. After about five weeks, when the salt concentration has been increased to about 16%, it is held at this strength until the pickless are used.

As postulated by DEMAIN and PHAFF (102) *Aerobacter* and lactobacilli predominate at the start of the fermentation. After five days the population of *Aerobacter* decreases rapidly owing to growth and acid production by the lactic acid bacteria (*Pediococcus cerevisiae*, *Lactobacillus brevis* and mainly *Lactobacillus plantarum*). Yeast population was shown to increase slowly, reaching a peak after about 16 to 18 days.

The possible sources of the softening agents are limited to those enzymes which are not inactivated by the acidity and salt content in the brine. For this reason the pectolytic activity of various organisms which develop during curing has been investigated. Further, it should be remembered that BELL, ETCHELLS and JONES (34, 35, 36) have found that cucumbers contain pectinesterase and polygalacturonase.

For some time, it was believed that pickle spoilage was due to pectic enzymes produced by members of the genus *Bacillus* (102). However, later it was found that the activity of such bacterial enzymes was inhibited by 7 % salt and that the bacilli need a pH of 5.5 or above; these conditions are believed too restricted to account for the outbreaks of pickle softening. Because yeasts possess high salt and acid tolerances, their production of pectic enzymes, as well by surface yeasts (239) as subsurface yeasts, could by very important.

HAMILTON and JOHNSTON (189) studied the polygalacturonase, polymethyl galacturonase and pectinesterase producing activity of a large number of isolates of bacteria and yeasts from commercial cucumber fermentations under simulated commercial conditions and found that this activity was not significant. Of the mould isolates, representatives of 10 genera, possessed moderate to high pectolytic enzyme-producing activity.

According to BELL, ETCHELLS and JONES (35), the polygalacturonase-like enzyme from cucumber fermentations and the fungal polygalacturonase from Pectinol reacted alike (temperature optimum 30° C, pH-optimum 4.0, no inactivation by increasing salt concentration up to 21 %). BELL and ETCHELLS (40) treated experimental packs of pasteurized cucumbers with fungal polygalacturonases from different sources under controlled conditions with respect to temperature, pH, acidity, salt concentration and in absence of microbiological development; it was revealed that as the salt content of the cucumbers increased, the firmness likewise increased to a first order reaction.

The work of BELL, ETCHELLS and JONES (159) showed moulds to be the real agents of softening during curing. The fungi were found to enter the fermentation process mainly via the heavily contaminated cucumber flowers which may remain attached to the fruits. ETCHELLS ET AL (161) made detailed investigations into the seasonal fungal populations occurring in cucumber flowers, ovaries and fruits and found that 34 genera out of a total of 72 moulds produced pectolytic and cellulolytic enzymes and included the moulds most frequently recovered.

Removal of flowers resulted in a low enzyme activity and firm fruits in the brine. The incidence of softening may be reduced also by draining the original brine after 36 hours (159). This is an indication that development of moulds takes place during a short time or that the enzyme activity is derived from moulds growing in the dead flowers and that pectolytic enzyme production mainly occurs prior to and not during brining, which has been postulated by HAMILTON and JOHNSTON (189). Moulds do not survive during a long time the unfavourable anaerobic conditions during curing. ETCHELLS ET AL (160) and BELL ET AL (39) showed that the pectolytic and cellulolytic activity in the brine can be inhibited by tannin-like substances from Scuppernong grape leaves or by the us of a brine extract of sericea (41a).

Some cases of the softening of gherkins has been reported. HAVAS (195) proved that this breakdown is related to breakdown of pectic substances during curing.

As has been mentioned for cucumbers and gherkins, olives must be stored in brines also to insure adequate yearly production of canned ripe olives. During the first weeks the olives undergo a spontaneous fermentation usually dominated by lactic acid bacteria. CRUESS (91) found participation of pectolytic enzymes in the stem-end softening of olives. BALATSOURAS and VAUGHN (30) studied the pectolytic activity of 35 moulds

and lactinomycete from surface growth of olive brines. Except for two isolates, all organisms possessed the ability to soften a pectate medium.

During production of sauerkraut by a fermentation process of shredded white cabbage the crisp texture of the cabbage is sometimes decreased.

In the production of sauerkraut the shredded cabbage is mixed with some salt; the final concentration of salt may vary from 1.5 % to 3.0 %, according to individual procedures. A salt percentage of 2.0–2.5 % has shown to be optimal. After mixing with salt the cabbage is pressed to promote anaerobic conditions, whilst the leakage of juice from the cabbage is enhanced by the salt.

In the beginning of the fermentation process the growth of representants of bacteria from the *coli-aerogenes* group and *Leuconostic mesenteroides* and *Pediococcus cerevisiae* is rather important, but during later stages of fermentation when the pH is further decreased, the predominating bacteria are *Lactobacillus plantarum* and *Lactobacillus brevis*. In all cases a population of yeasts will be present also (360).

WIERINGA (518) has proved that the occurrence of softening of sauerkraut is often related to a low salt concentration. This deterioration showed to be promoted also when at the start of the fermentation process the temperature was above 23 °C.

According to the experiments of DURACH (148) the softening of sauerkraut should be caused by innate pectolytic enzymes from the cabbage (*Brassica oleracea* var. *capitata*), which are inhibited by salt. In relation to this it should be mentioned that HOOGZAND and DOESBURG (223) showed also the presence of pectolytic enzymes in cauliflower (*B. oleracea var. botrytis subvar. cauliflora*). DURACH (148) has postulated that the activity of this pectolytic enzymes depends on the varieties used, the early varieties showing a higher activity than the late ones. To prevent the incidence of softening during sauerkraut fermentation, it is important that the salt is distributed homogeneously throughout the product.

Influence of heating

The changes in firmness of plant tissues during heating are partially induced by alterations in cell walls.

In many cases, attempts have been made to explain the influence of cooking on firmness and texture of plant tissues from changes which occur in the pectic substances (45, 137, 167, 286, 302, 303, 373, 396, 432). It will be shown, however, that not in all cases could the behaviour of pectic substances account for the alterations in firmness and texture which take place during heating and which usually are expected to depend on changes in pectic materials in the cell walls.

Investigations were made to study the relation between firmness of plant tissues and behaviour of pectic substances when cooking these tissues at different pH-values (137, 138, 141, 302).

DOESBURG (137, 138, 141) measured the firmness of 1 cm-cubes of tissues of beets, turnips, potatoes and unripe apples after cooking at pH-values ranging from $\pm$ 3.0 to $\pm$ 6.5. After cooking, the firmness of the cubes was measured with the I.B.V.T.-hardness meter (135, 141), shown in Fig. 49; with this instrument the force needed to press the plant tissues through a screen is determined. The content of soluble pectic substances was estimated also.

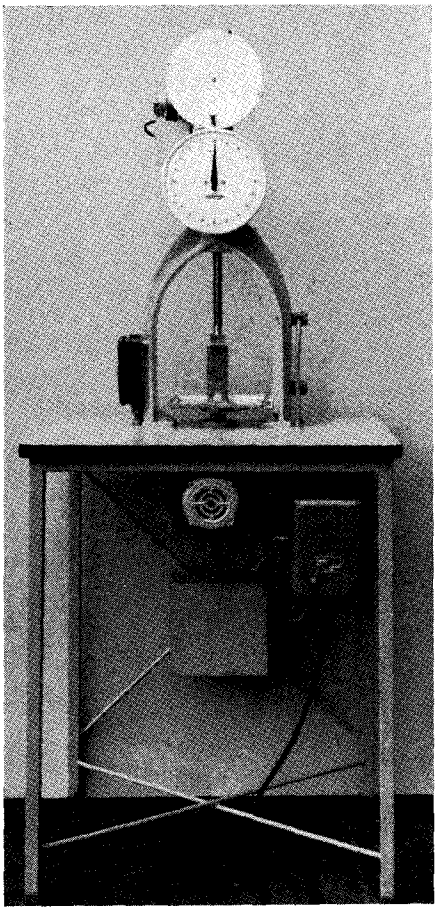


FIG. 49. I.B.V.T.-hardnessmeter for measurement of firmness of plant tissues (Doesburg and Grevers, 141; Doesburg, 135).

Beets, turnips, potatoes and unripe cored apples were peeled and cut into 1 cm-cubes. Three one-hundred g lots of the diced products were placed in 300 ml aliquots of 0.5 % sodium citrate solution which were treated with a few drops of conc. HCl or NH_3 solutions to give pH-values ranging from 3.0 to 6.5. With the use of vacuum, the cubes were impregnated with the surrounding solutions at different pH-values which were maintained constant for three hours by the addition of more HCl or NH_3 solution. The cubes were then cooked for 10 minutes in the same solutions and finally cooled in air, after spreading on a screen and firmness measured with the I.B.V.T.-hardness meter.

For the determination of soluble pectin, the cubes from every treatment were ground in a waring blender after being suspended in 600 g water. The pH of the resulting product was adjusted with NH_4OH or HCl to pH 4.5 and the soluble pectic substances in the filtrate determined.

The results, obtained with the various products were identical. In a somewhat different experiment, similar results were found with cauliflower. The effect of cooking on texture of turnips is presented in Fig. 50. The pH-values are those of the product after cooking, which caused a slight change of the pH before heating.

The fact that the same phenomena were found in potatoes, beets, turnips, apples

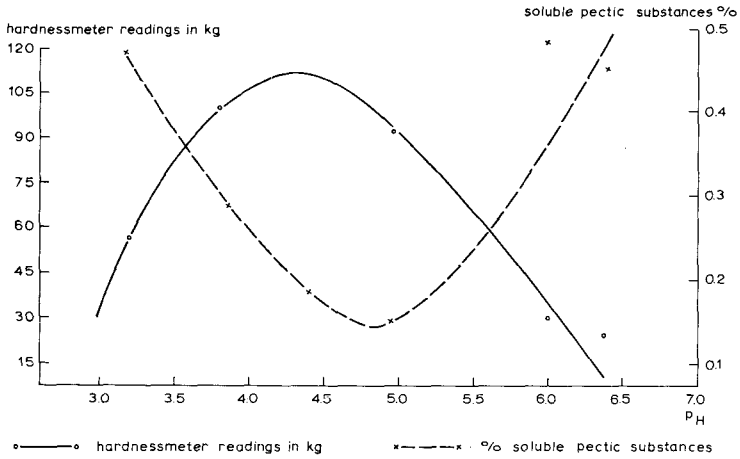


FIG. 50. Relation between firmness and formation of soluble pectic substances during cooking (15 min.) of plant tissues (Doesburg, 137).

and cauliflower is an indication of an identical structure of cell walls in the mainly parenchymous tissues from various organs of edible crops.

Plant tissues cooked at pH 4.0–4.5 are firmer than after boiling at higher or lower pH-values. Generally speaking, the decrease of firmness at higher or lower pH-values corresponds with an increase in soluble pectic materials. From this formation of soluble pectic substances it is evident that in both cases decomposition of protopectin takes place, but these effects depend on different causes.

As shown in Fig. 27 the depolymerization of pectins cooked at pH 3.0–4.0 is very slight, whilst, at higher pH-values a strong depolymerization is occurring. For this reason it may be expected that, by contrast with cooking at the high pH-range (4–7), the boiling at lower pH-values will result in the solubilization of pectic materials of a rather high molecular weight. This is confirmed by the viscous character of such products, e.g. apple sauce, after cooking. As has been pointed out by DOESBURG (137) the formation of soluble pectic substances during cooking in acid environment must be attributed mainly to the hydrolysis of other cell wall constituents, particularly hemicelluloses which are attached to the pectic substances by covalent or secondary bonds. According to literature (20, 211, 230), hemicelluloses are much more sensitive to hydrolysis in acid milieu than are pectic substances.

During cooking in low-acid or neutral milieu (pH 4–7), the formation of soluble pectic substances can be explained by the depolymerization of pectic materials, which is rather slight at pH $\pm$ 4 but strongly increasing with elevated pH-values (see Fig. 27); the rate of depolymerization is increased with increase of degree of esterification. Such a relation between depolymerization and solubilization has also been shown by the depolymerizing action of pectolytic enzymes (183), γ-rays (306) or by a similar influence of a milieu of ascorbic acid and H_2O_2 (183, 258). At more elevated pH-values the stronger depolymerization may cause a decrease of the total amount of pectic substances. This was observed by SIMPSON and HALLIDAY (432) in the case of cooked vegetables. In contrast to the opinion of SIMPSON and HALLIDAY, the author concludes that, during cooking, at most no more than slight changes in the cellulose fraction will

occur. STERLING (456) has attributed the ease of staining of cellulose after cooking to the degradation of protopectin (see also p. 14).

As shown in Fig. 50 the maximum firmness of cooked tissues does not correspond with the pH-values at which the minimum amount of soluble pectic materials can be found. It must be assumed that, during cooking at pH-values just above 4, the structure of protopectin is already weakened somewhat by an onset of depolymerization, however, not yet strong enough to cause solubilization.

Another cause of the formation of soluble pectinic acids may be an increased solubility at higher temperatures, as has been postulated by FREEMAN and RITCHIE (167). However, from the data presented in Fig. 50 it is evident that this influence is quite small, since the quantity solubilized by this factor at most equals the minimum quantity of solubilized pectic materials which is found at pH $\pm$ 4.8.

The solubility and swelling of pectic substances are influenced also by their degree of esterification and the amount of available calcium. The amount of active calcium is governed by the calcium content (naturally present or added), and the amount of calcium-binding constituents in the tissues. As shown in Fig. 7 and Fig. 12, above pH $\pm$ 4 the swelling is promoted by an increase of pH, which also causes a decrease of firmness of tissues.

From the foregoing discussion it will be clear that there is a marked relation between behaviour of pectic substances and firmness of plant tissues cooked at different pH-values. Several factors are summarized in Table 9.

TABLE 9. *Factors influencing the relation between the behaviour of pectic substances and firmness of plant tissues during cooking at various pH-values.*

pH	Influence of cooking
4–3	increased solubilization of pectic materials, presumably by stronger breakdown of other cell wall polyoses with decrease of pH.
4–7	increased solubilization of pectic materials by stronger depolymerization of pectic substances with increase of pH; pectic substances are more sensitive to breakdown when their degree of esterification is higher.
4–7	increased swelling of pectic substances; the effect is enlarged by increase of pH and decreased by the presence of active calcium.
3–7	influence of degree of esterification of pectic substances. Depolymerization during heating at pH 4–7 is restricted when the degree of esterification is low. Pectic substances having a low degree of esterification are insoluble in acid milieu or their solubility at higher pH-values is decreased by the calcium content of tissues.
3–7	influence of the calcium, which precipitates pectic substances with a low degree of esterification and reduces the swelling of all pectic substances.
3–7	influence of calcium binding agents, which are reducing the amount of active calcium and promote swelling or solubilization.
3–7	influence of greater solubility of pectic substances which arises during heat treatment.

Though a relation between behaviour of pectic substances and firmness of plant tissues after cooking can be shown, many differences in firmness and texture between cooked products from various varieties or sources of the same products with approximately the same pH, cannot be explained on this basis.

It is known that 'sweet' apples, characterized by a low acid content (pH $\pm$ 4), show a poor saucing quality, which may be understood from the foregoing discussion. However, smaller differences of saucing quality can be observed also when the same property of high-acid apple varieties is considered. Within this group, REEVE and LEINBACH (396) did not find any relation between the saucing quality, contents of insoluble pectic substances or pH. According to REEVE (393), the observed differences are to be attributed to unknown differences in cell wall structure. KERTESZ, EUCARE and FOX (265) found differences of cellulose content between hard-cooking and soft-cooking apples.

The saucing quality of apples is mainly dependent upon the separation of cells. In the finished product, the liquid-solids separation caused by a flow of liquid under gravity, may have a disadvantageous effect on the quality. TOLDBY and WILEY (477) studied this phenomenon, which they called *lyophoresis*. The lyophoresis increased with the maturity of the fruits used; it was shown to be closely related to the viscosity of the free liquid from apple sauce which is almost completely determined by the pectin and starch contents and the pH. Addition of acid to sauces made from fully mature apples has a tendency to reduce lyophoresis whilst it was increased by addition of acid to sauce of immature apples.

In some other products small quantities of lignin which have been revealed by means of new more sensitive methods, might influence the behaviour of other cell wall compounds during boiling (31, 229).

The cooking quality of potatoes (sogginess or mealiness) was intensively studied by BETTELHEIM and STERLING (44, 45, 461). The texture of cooked potatoes was found to be closely related to their starch content and, for this reason, also with the specific weight of the raw materials. BETTELHEIM and STERLING (45) found no clear relationship between behaviour of pectic substances and the texture of cooked potatoes. Similar conclusions were reached by FREEMAN and RITCHIE (167) and PERSONIUS and SHARP (372, 373). In contrast to PERSONIUS and SHARP (372), STERLING and BETTELHEIM (461) concluded that gelatination of starch is the main factor for cell separation during cooking of potatoes.

REEVE (392) studied the relation between histological characteristics and texture in the seed coats of peas. The middle lamella in seed coats of young peas are mainly composed of pectic substances but later during maturation they are encrusted with pentosans. In the later stages, REEVE did not find an effect from various extractants of pectic substances. BOGGS, CAMPBELL and SCHWARTZE (56) have reported that tough pea seed coats could not be tenderized by boiling for 15 min.

Special attention should be paid to the influence of calcium-binding substances upon the cooking quality of various products. This property of the anionic buffer is of great importance for the behaviour of low-acid (pH 4–7) plant tissues which contain pectic substances of a rather low degree of esterification, since the depolymerization of polygalacturonic chains during heating will be limited because of their low methoxyl content. In these cases, the swelling and solubilization will depend strongly on the contents of available calcium. When the cells are killed, calcium-binding compounds (e.g. oxalic acid, citric acid) from the anionic buffer in the cells may act upon the calcium in the cell walls and cause a decrease of the available calcium, which promotes the swelling and solubilization of the pectic materials.

MATTSON (302) showed that the cookability of cooked field-dried yellow peas is related to their natural content of phytic acid (inositol phosphoric acid), which is a Ca- and Mg-precipitant; its precipitating power is greatly increased at boiling temperature. Further studies and discussions on the influence of phytic acid have been given by MATTSON ET AL (303), FOWLER (165, 166), WAGER (501), DICKINSON (120) and ANTHISTLE (8).

Phytic acid combines preferentially with calcium to form phytin. When phytin is destroyed, alkali earth metals are liberated which may then form pectates.

FOWLER (165) has shown that phytin occurs in a pure state in peas. He studied the changes in inorganic orthophosphate in relation to phytin formation in maturing pea seeds (166). The changes appeared to take place in three stages. In the first stage, the orthophosphate content was initially high and fell rapidly, while the phytin content rose. In the second stage the orthophosphate content increased and the phytin fell sharply, but began to rise again at once. In the final stage the orthophosphate fell and the phytin slowly increased.

According to MATTSON ET AL (303), soils rich in soluble phosphorus yield mature peas which are rich in phytic acid also. Different varieties of peas on the same soil differ somewhat in phytic acid content and in cookability, but these differences are not outstanding. Rather dry growing conditions may decrease the phosphate intake and cause a poor cooking quality. Unripe dried peas did not swell when soaked in water for 18 hours; when a short preliminary scalding in boiling water was applied the swelling was normal and the cookability depended on the phytic acid content.

FOWLER (166) has suggested that the hardening of peas during storage may be due to enzymic breakdown of phytin and liberation of Ca- and Mg-ions. Since the optimum temperature for phytase activity is about 55° C, this activity could also occur during soaking at relatively high temperatures or even during the early stages of cooking.

CREAN and HAISMAN (90) found that, during cooking of peas in extremely hard water, only 60 % of the total phytate in the peas was complexed and that insoluble phytates account only for a proportion of the absorbed ions. For this reason they concluded that the influence of phytate ions on texture is small.

WAGER (501) studied the influence of phytic acid on potatoes, and concluded that differences in texture between new and old potatoes depend on the properties of the materials binding the cells together rather than on the phytic acid content.

Firming of heat processed and frozen products

Since the swelling of pectic substances is reduced and pectinic acids with a low degree of esterification are precipitated by addition of calcium salts, it is possible to use calcium salts as firming agents for plant tissues.

When high-methoxyl pectinic acids have been solubilized by the ripening of fruits or by a heat treatment, it is to be expected that these materials have no significance in relation to the tissue firming action by calcium salts, since they are not precipitated. By contrast with soluble high-methoxyl pectinic acids, the pectinic acids with a low degree of esterification are most sensitive to these firming agents. LOCONTI and KERTESZ (286) identified calcium pectate as the tissue-firming compound formed by treatment of tomatoes with calcium chloride.

When pectic substances with a low degree of esterification are not present the firming action of calcium will be smaller or even undetectable (223).

JOUX (251) studied the influence of pectic substances on firmness of canned apricots. From his experiments, he concluded that the protopectin fraction was the most important in relation to firmness of fruits and that the influence of other fractions, with a low or high degree of esterification was negligible. For this reason it is advantageous for apricots not to be heated to temperatures higher than 80–85 °C, to prevent decomposition of protopectin. After such a moderate heat treatment apricots could be firmed by application of calcium salts (251, 252).

When over-ripe apples were used (149), or apples held in cold storage during four months (86), addition of Ca-salts did not yield slices with an acceptable firmness. The failure of the action of firming agents under these conditions may be regarded also as a result of protopectin decomposition during ripening. The pectic substances with

a high degree of esterification, present in apples, are not flocculated by calcium salts.

The proportions of water-soluble, versene-soluble and versene-insoluble fractions were examined in six varieties of peaches. The versene-soluble fraction was most closely related with firmness retention of the canned product (429a).

Experiments on firming of canned or frozen products have been made with canned tomatoes (149, 286, 384), canned apricots (251, 252), frozen or heat-processed apples (11, 86, 149, 221), mango fruits (391), cauliflower (208, 223), potatoes (11, 403), sweet peppers (224) and other products (peaches, cherries, currants, fruit pulps, olives, pickles), as has been reviewed by Durocher and Roskis (149). Calcium treatment of strawberries, preserved with sulphur dioxide, has been reported before (see p. 99).

The manner of application of calcium salts depends on the commodity. Calcium treatment may be carried out on the raw or peeled product by dipping it in the salt solution, eventually accompanied by the use of vacuum to facilitate the penetration of the solution, but the treatment may be applied also during blanching or by addition of calcium salts to the can contents.

According to ARCHER (11), calcium salts are often used for firming of canned tomatoes and potatoes, but less often for apples. Nevertheless, in recent experiments by Archer (11) and COLLINS and WILEY (86), the use of calcium salts was found to be valuable for firming of apple slices. The benefit of calcium treatment of canned mango fruits was very slight or absent as has been concluded by RANGANA, SASTRY and SIDDAPPA (391).

Much attention has been paid to the effect of various calcium salts and their manner of application.

Several trials showed that in apple products Ca-malate, -gluconate, -phosphate and -citrate have the same reinforcing effect as calcium chloride. The effect of the lactate was somewhat better than that of the other salts used (149, 221, 259). HOLGATE and KERTESZ (221) noted that, in some experiments, the amount of calcium in the lactate treated apples was higher than in any of the samples dipped into solutions of the other calcium salts. COLLINS and WILEY (86) proved also that calcium lactate was more efficient for firming thermal processed apple slices than calcium gluconate. Magnesium salts were inefficient in promoting firmness of apple slices.

PORRETTA and CASOLI (384) made experiments with various Ca-salts with peeled tomatoes. The best results were obtained with calcium chloride or phosphate, whereas the use of calcium citrate was not effective at all.

HOOVER (224) compared the effect of calcium hydroxide, calcium chloride and calcium sulphate on firmness of canned green and red sweet bell pepper, *Capsicum frutescens*. Calcium hydroxide was very effective as a firming agent when it was applied to fresh peppers prior to heat processing and was the most effective firming agent. Similar results have been obtained by use of calcium hydroxide in cauliflower before canning as shown by HESS (208).

It is difficult to understand why calcium lactate yields better results than other firming agents, when used for firming of apples. The superior effect of calcium hydroxide treatments of raw products may be related to the high pH of the solution, which may cause some activation of pectinesterase or alkaline saponification of pectic substances in the tissues.

Application of calcium chloride for firming of apple slices (149, 221), sweet peppers (224), mango fruits (391) or cauliflower (208) caused a bitter after taste. No objectional flavours of any kind in apple products could be observed with calcium phosphate, malate or lactate (221), but a harmful effect on flavour resulted from the use of calcium citrate (149). When treating canned cauliflower (208) the least objectionable effect on flavour occurred with calcium hydroxide.

Firming of canned fruits by calcium treatment results in a greater drained weight of these products. As postulated by STERLING (457, 460), the drained weight depends also on absorption of sugars by cell wall materials. The absorption of sugars by cell walls has been proved by STERLING and CHICHESTER (460) by use of radioactive sugars. The higher concentration of sugar in the cell walls should permit water to move into them and to produce a more hydrated condition there by which cellular volume and drained weight are increased. Further, it must be noted that, as a result of the dehydrating effect of sugars in the solution, fruit canned in heavier syrup will be firmer than fruits canned in a lighter one.

Since pectic substances with a decreased degree of esterification show an increasing sensitiviness to action of calcium salts (naturally present or added) the firmness or toughness of plant tissues will be promoted as a result of the action of pectinesterase before preservation.

As shown by BUCH, SATORI and HILLS (65), such an action of pectinesterase seems to be involved when raw red tart cherries are allowed to stand before being canned, either with or without having been previously bruised. During the ageing period a small portion of the pectic substances was completely demethylated. The increased firmness of aged cherries persists through the canning process, as shown in Fig. 51. WHITTENBERGER and HILLS (513) reported also that ageing in air or water before canning increased the drained weight and firmness of the cherries. When the cherries are bruised before the ageing period this effect may be even more pronounced.

As shown by DOESBURG (132), HOOGZAND and DOESBURG (223) and VAN BUREN ET AL (491) pectinesterase in vegetable tissues can be activated by heating to 60–80 °C. This effect has been used for the firming of canned cauliflower (222, 223) and to prevent the sloughing of frozen or canned snap beans (253, 345, 437, 490, 492). Selective

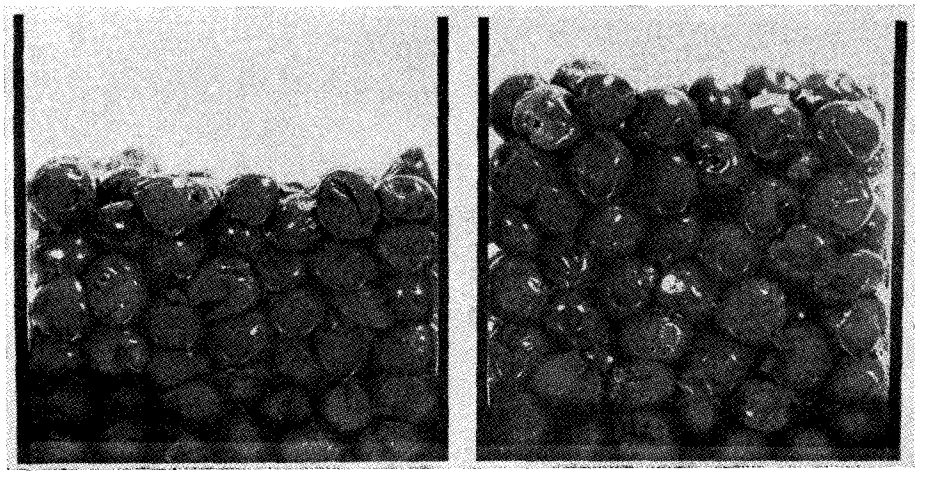


FIG. 51. Effect of bruising and ageing on firmness of red tart, pitted cherries. Each jar contains pitted canned cherries, equivalent to 450 g of fresh, whole cherries. Left: control processed immediately after harvesting. Drained weight = 68%. Right: cherries bruised twice by dropping from a 2-ft height, allowed to stand 8 hr at room temperature, and then 16 hrs. in ice water before being processed. Drained weight = 74% (Buch, Satori and Hills, 65).

demethylation, resulting from 30 sec. blanching at 100°C and 10 minutes holding time, increases the firmness of canned tomatoes (226a).

By contrast with the common high temperature – short time (HTST) blanching the firmness of canned cauliflower can be promoted by a low temperature – long time (LTLT) blanching. Nevertheless the effect of such a LTLT-blanching proved to be insufficient to yield a favourable result after preservation by a botulinum cook. Therefore LTLT-blanching has to be combined with a calcium treatment and the addition of a small amount of citric acid to decrease somewhat the pH of the canned cauliflower, which has also a firming effect (see Fig. 50).

Further experiments (208) with various blanching and other pre-treatments showed that calcium and acid addition have to be applied in all cases to yield a firm canned cauliflower.

The sloughing and splitting of snap beans can effectively be decreased by LTLT-blanching (253, 345, 437, 490). The favourable influence of this treatment and a holding period after blanching is related to pectinesterase activity (253, 491, 492). According to KACZMARZYK ET AL (253) sloughed tissue of White-seeded Tendergreen beans consists of the epidermal layer, as shown in Fig. 52.

VAN BUREN and PECK (493) proved that Tendercrop snap beans, grown in quartz sand with different nutrient solutions, had less tendency to slough and split when higher Ca-levels in the nutrient solutions were applied. Similar trials by SAYRE and NEBEL (417) and REEVE (392) showed that a high Ca-level in the nutrient solution increased the toughness of pea seed coats.

SISTRUNK (436a) showed that post-harvest storage of snap beans at high temperatures (55° and 85°F) increased sloughing; the temperature of blanching influenced the amount of sloughing after storage at 35°, 55° and 85°F. Fiber content, including lignin and other highly insoluble constituents, increased during storage.

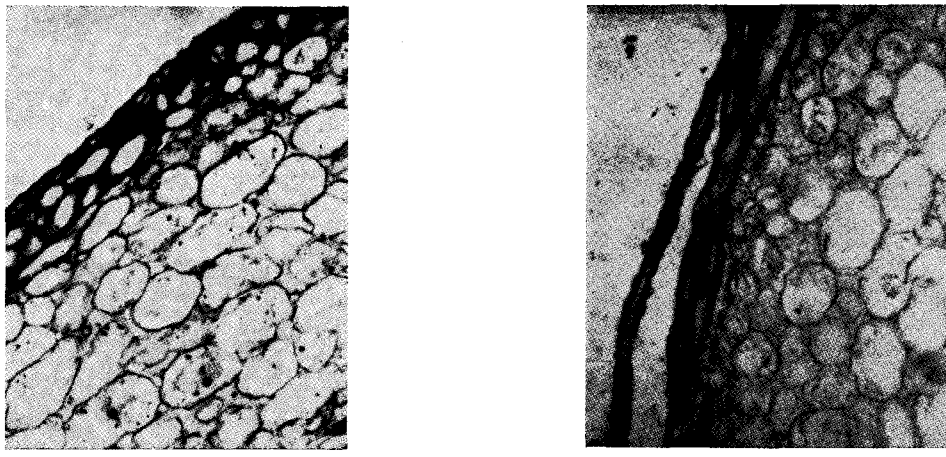


FIG. 52. Photomicrographs of canned bean cross sections, stained with ruthenium-red. Left: LTLT-blanched bean. Right: sloughing bean, canned without firming pre-treatment (Kaczmarzyk, Fennema and Powrie, 253).

Rehydration of dehydrated products

When put into water, dehydrated vegetables and fruits absorb water and soften. The folded, wrinkled walls tend to straighten out during rehydration and to return to their original shape, thus sucking water into the cavity of the cell.

As postulated by GANE and WAGER (172) the stretching of cell walls should be due to their natural elasticity. However, STERLING and SHIMAZU (462) pointed out that the property of elasticity of cell walls is a function of the crystalline content of the wall; they showed that the more highly crystalline and elastic the cell wall, the poorer the volume of reconstitution. According to STERLING and SHIMAZU (462), volume recovery is promoted by a higher amorphous content of the cell wall components, which promotes the swelling and re-establishment of original shape of cell walls during rehydration. In this connection, the amount and nature of the pectic substances will also be involved in the behaviour of dehydrated products. OTT and BALL (361) and SNYDER (443) showed that there is a correlation between the water absorption and content of pectic materials of seed coats of *Phaseolus vulgaris*. BAKER and MURRAY (26) indicated that the viscosity of extracted pectinic acids from dehydrated apples, peaches and carrots may be an index to their quality. This relationship was not found with apricots.

The water adsorption of pectic substances has been studied in model systems.

Structural changes in the polygalacturonide chains during water adsorption were studied by BETTELHEIM and STERLING (46). Water-vapour isotherms of highly esterified pectins, sodium pectate and pectic acid were determined. The interchain separation, as measured by changes in the equatorial spacings of X-ray reflections, followed the shape of the adsorption isotherms. In esterified pectin and sodium pectate the fiber period increased until five molecules of water per three galacturonide residues are adsorbed, thereafter remaining constant. The fiber period of pectic acid appeared to be constant throughout water adsorption.

SHIMAZU and STERLING (430) compared the behaviour of cellulose preparations (containing some resistant hemicelluloses) from the phloem of carrots and from cotton fibres, and a calcium pectinate gel during dehydration at two different rates and during six months storage. In all cases, dehydration resulted in an increase in the crystalline content of the samples, rapid dehydration seeming to produce somewhat greater crystallinity than slow dehydration. All materials became more crystalline on standing. After six months, per sample type, the products resulting from both dehydration methods, attained the same crystallinity values. Ca-pectinate had the highest amorphous content.

BAKER, KULP and MILLER (29) studied the relation of pectic substances in relation to dehydration and rehydration of simulated fruit preparations.

As far as swelling of cell walls is dependent on the presence of pectic substances it may be expected that this swelling is promoted by the presence of pectic materials of high molecular weight and high degree of esterification and that swelling is diminished by a decrease of pH or the presence of polyvalent cations, especially calcium.

However, other factors will also be involved. STERLING and SHIMAZU (462) dehydrated blanched and unblanched dices of carrot phloem and stored these products for periods of up to six months. The rehydrated volume was found to be always greater for blanched dices than for the unblanched material whereas, in all samples, the rehydrated volume decreased as a result of storage. The blanching effect was still readily perceptible after six months (see Fig. 53). A higher crystallinity was found in unblanched than in blanched samples and a continuous increase in crystallinity was

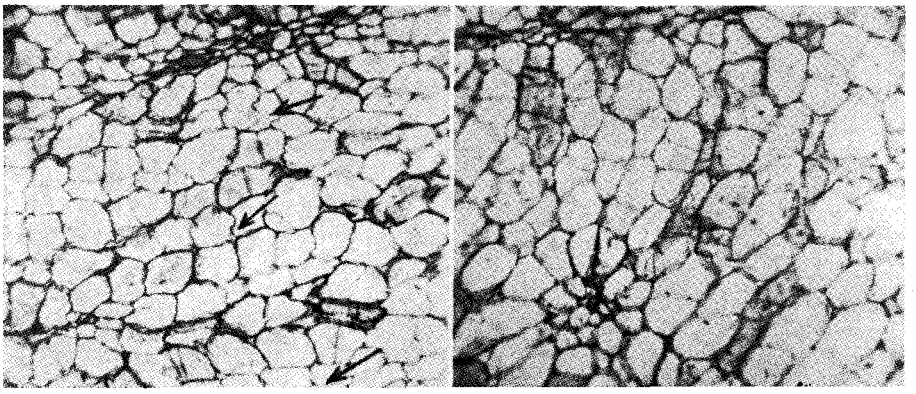


FIG. 53. Cross section through secondary phloem of rehydrated unblanched (left) and blanched (right) carrot after six months of storage. Arrow-heads are located near region of marked cell-wall wrinkling in unblanched tissue. Cell walls in the whole section of unblanched tissue show some degree of wrinkling or folding, which is not found in the blanched tissue (Sterling and Shimazu, 462).

noted in all samples as storage time increased. STERLING and SHIMAZU (462) concluded that blanching helps maintain the amorphous phase, which should hinder the crystallization process during storage. According to STERLING and SHIMAZU, this preventive effect should be related to further molecular separation in the amorphous regions with an increase in the extent of molecular disorder or the blanching process might cause some hydrogen bond rupture in crystalline mycelles.

DOESBURG (138) paid attention to the result of inactivation of pectic enzymes during blanching. When products are dehydrated without foregoing heat treatment, it is to be expected that partial saponification and breakdown of polygalacturonic chains by pectic enzymes occur during dehydration and perhaps also during storage. When, in contrast with the results of a normal blanching process, the pectinesterase is not destroyed, but is instead activated by a previous low temperature – long time (LTLT) blanching process, the swelling ability of dehydrated products will be reduced (see Fig. 11). A similar result is to be expected when polygalacturonases have not been destroyed by a previous blanching process.

CRAFTS (89) declared that prolonged blanching tends to reduce the ability of cell walls to absorb water. In similar experiments, the author (140) has found the same influence of prolonged blanching on dehydrated carrots. However, the influence of prolonged heating prior to dehydration is not found in all cases; FENNEMA and WECKEL (163) found no significant differences in rehydration ratio of snap beans which were pre-cooked for 5, $7\frac{1}{2}$, or 10 minutes. As far as pectic substances may be concerned in the effect of blanching time on vegetables, it should be remembered that, during heating in a weakly acidic or neutral environment, a rapid depolymerization of pectinic acids takes place. From this point of view it must be concluded that the blanching treatment should not exceed the time required for effective inactivation of pectic enzymes.

The influence of enzymic and non-enzymic browning reactions upon the behaviour of pectic substances in dehydrated products is discussed on p. 131.

Jellying power of fruits

For several reasons, the jellying properties of pectic substances in fruits are very important in relation to jam manufacture. The formation of a gel in jams improves their organoleptic quality and spreadability. Furthermore it may prevent fruits from floating and promote an even distribution of fruits or pieces of fruits in the finished products.

For the formation of a gel, it is necessary for the pectins to be solubilized. In most kinds of mature fruits, the greater part of the pectin is solubilized during the ripening process, but in other ripe fruits an important part is still bound in the tissue. In these latter instances the fruits (apples) or fruit parts (citrus peels) have to be previously steamed or extracted to promote solubilization of pectins (see also p. 99). The extraction of pectins during boiling, for jam manufacture, is not always complete. McGREGOR (332) found that jam boiled by vacuum methods required addition of more pectin to obtain a set comparable with jam produced in open kettles. The author considers that this effect is due to incomplete release of pectin from the fruit boiled at lower temperatures under vacuum. However, some pre-setting, more rapidly caused at these lower temperatures, may be involved also (p. 39).

The jelly grade of fresh fruits may be defined as the number of grams of sugar that can be converted into a jelly of standard strength by the pectin extracted from one gram of these fresh fruits.

As pointed out by KERTESZ (259), cherries, apricots, pears, blueberries, nectarines, peaches, raspberries, strawberries and pineapples are low in useful pectins which also should often be true of blackberries, grapes, loganberries, plums (except Damson). Fruits such as apples, cranberries, currants, Damson plums, guavas, gooseberries and quinces are found to be rich enough in pectin to make preserves with but a small amount (or none) of added pectins.

JAKOVLIV and COLPE (232) stated that, of Belgian fruits, gooseberries, black and red currants and apples have good jellying power, plums and mirabelles a moderate power and strawberries, bilberries and cherries a very poor jellying power. This property may vary from year to year, especially in red currants and plums.

Seasonal variations and differences between different varieties of one kind of fruit have also been found by DOESBURG (139). The mean jellying power of different kinds of fresh fruits, grown in the Netherlands, is given in Table 10 (139).

Deviations of about 25% from the mean values have been found. In addition to the factors mentioned above, the jellying power of fruits is influenced by their stage of ripening. In many fruits, the jellying power is decreased during ripening.

From Table 10 it can be concluded that some fruits are so rich in pectin content that they would give too firm jellies when higher fruit contents are used. In such cases, higher amounts of the fruits can be successfully used only when, in a portion of the fruit, the pectins have been degraded.

With other fruits, the addition of pectins and adjustment of pH is often necessary to produce a satisfactory gel.

The jellying power of fruit pectins is governed by their pectin content as well as by the degree of polymerization, degree of esterification and the acetyl content of these pectins.

TABLE 10. *Mean jelly grades, as determined with the Wageningen method (125), of various fresh fruits, grown in the Netherlands. Theoretical percentage of fruits in the jam, needed to produce a standard jelly.*

Fruits	Jelly grade	Theoretical percentage necessary for standard firmness
black currants	± 3.0	± 22 %
plums	± 2.5	± 26 %
gooseberries	± 2.0	± 32 %
apples	± 2.0	± 32 %
red currants	± 1.8	± 36 %
raspberries	± 1.0	± 65 %
strawberries	± 0.5	± 130 %
cherries	± 0.5	± 130 %

The degree of esterification usually decreases during ripening. In most cases, the degree of esterification varies from about 50–70%. Pectins with a high methoxyl content are present in apples and citrus peels; these methoxyl contents are only slightly affected during ripening. The pectins from apples in the period of commercial harvest may often result in a very rapid set, showing a degree of esterification of 80–82%, which is decreasing to about 75% during ripening.

The optimal pH-values for the production of good jams has been shown to be ranging from about 2.9–3.3. For the adjustment of pH, addition of some acid is often required, which depends on the acidity of fruits. In the cases of fruits with a high pH (>4) (pineapples and some varieties of cherries), it will be necessary that the acid is added before the addition of pectin (279), since the pectin is rapidly degraded during boiling at pH-values mentioned above.

It should be remembered that heating is not necessary for jelly formation, even with high-methoxyl pectins (see p. 35). JOHNSON and BOGGS (57, 237) described the manufacture of jellies and jams at room temperature. The products can be preserved during a long time by freezing without a loss of quality.

The influence of temporary preservation of fruits by freezing or sulphiting has been discussed extensively before (see p. 97), while the factors which govern the gel formation during boiling, cooling and filling of jars have also been reported earlier (p. 40).

Fruit juices

The behaviour of pectic substances and pectic enzymes play a very important rôle in fruit juice technology.

For the manufacture of clear fruit juices a breakdown of pectic substances will be needed to enable clarification and filtration, whereas in a number of cases the breakdown of pectic substances before pressing of fruits is necessary to produce an economic yield of juice.

In cloudy juices, especially citrus juices and tomato juice or tomato paste, it is essential that the original content of pectic substances of the freshly pressed juice

shall be retained throughout the production and shelf life of the juice to ensure cloud stabilization. Therefore, in these cases, innate enzymes of the fruits should be inactivated or inhibited as soon as possible.

The use of pectic enzymes

To produce an economic yield of juice from apples and pears, the use of pectic enzymes is not needed. The juice can be pressed immediately from the milled raw fruits.

Juices, pressed from ripe fruits without foregoing enzyme treatment may contain considerable amounts of pectic materials, which have to be removed for the manufacture of clear juices. DUDEN (147) indicated that the amount of pectic materials in apple juice is strongly dependent on the manner of removal of juice from the milled fruits. When the juice is centrifuged out immediately after the milling of apples, the amount of pectic substances showed to be much lower. In these cases the juice could be filtered without enzymic pre-treatment, yielding a clear or weak opalescent product. According to DUDEN it is essential that the juice is removed from the milled apples without any delay.

In order to enable filtration in the manufacture of brilliant clear juices, pectic enzyme preparations are added to normally pressed apple juices, since the clarification of fruit juices by their naturally occurring enzymes proceeds but slowly (74, 400).

According to CHARLEY (74), the removal of pectic substances is certainly not the only factor which is involved in the clarification process. The reduction of viscosity allows the sedimentation of finely divided materials. Further the removal of pectic substances, which may be regarded as a stabilizing factor for other colloids, causes a deposition of the latter substances.

Several authors have studied the assay of commercial pectic enzyme preparations (271, 400, 402, 423, 425, 504).

WEBER and DEUEL (504) used a viscosimetric assay of commercial preparations. In this method the enzymes are allowed to act on pectin solutions under standard conditions. After a certain time the decrease of viscosity is calculated as a percentage of the maximum decrease of viscosity, which has taken place when pectic substances are completely decomposed.

$$\text{Decrease of viscosity } (=DV) = \frac{Va—V}{Va—Vo} \times 100\ \%.$$

Va = viscosity in seconds of pectin containing solution when no enzyme is added.
V = viscosity in seconds of the solution with enzyme after a definite time.
Vo = viscosity in seconds of a sample in which all the pectin has been destroyed.

REID (400) mentioned a viscosimetric method in which the unknown preparations are compared with a standard enzyme preparation and showed that the relationship between DV and the logarithm of the amount of enzyme is linear.

SCHUBERT (423) proved that the clarifying action of pectic enzyme preparations depends on the decrease of viscosity of the product. Since different types of enzymes may be present and the degree of esterification of the pectic substances may be different also, the effect of a pectic enzyme preparation on various substrates may vary. For these reasons, it was concluded by SCHUBERT (423) that determination of the activity of depolymerizing enzymes as well as pectinesterase activity is important for the estimation of the clarifying action of enzyme preparations in various products.

According to RENTSCHLER (402), the action of commercial enzyme preparations in apple juices must be judged under practical conditions by addition to freshly pressed juice together with the use of varying amounts of gelatin, which is known to promote clarification.

In contrast to hard fruits, soft fruits cannot be pressed easily before a removal of pectic substances has been carried out to reduce the viscosity of the crushed fruits.

As postulated by CHARLEY (74) the use of such commercial enzymes followed earlier attempts to allow the natural enzymes and these produced during a slight alcoholic fermentation to effect sufficient degradation of pectic substances to enable efficient pressing. This was found to be entirely impracticable on account of the long time needed for removal of pectic substances during which period fermentation became excessive. Probably the ultimate degradation of pectic substances achieved by such a process was due both to the innate fruit enzymes and to others produced by the yeasts.

To reduce the dangers of alcoholic fermentation and to speed up the enzyme treatment (which is called usually also 'fermentation'), it is important that relatively high temperatures are applied during the enzymic pre-treatment of the milled soft fruits as has been shown by KREBS (272, 273, 274), KOCH (270) and HEIMANN, WUCHERPFENNIG and REINTJES (196). According to CHARLEY (74), the dangers of alcoholic fermentation are progressively reduced when the temperature is increased above 45 °C, but the possibilities of further elevation of temperature are limited on account of excessive inactivation of pectolytic enzymes. Therefore it will not be possible to use temperatures above 55–60 °C.

When, in some cases, the complete removal of pectic substances is not considered to be advantageous, the enzymic fermentation may be carried out at rather high temperatures (above 55 °C) so that sufficient degradation of pectic substances will take place to facilitate the pressing operation, but some residual pectic materials are not decomposed. These residual pectic substances lead to a final product with a certain

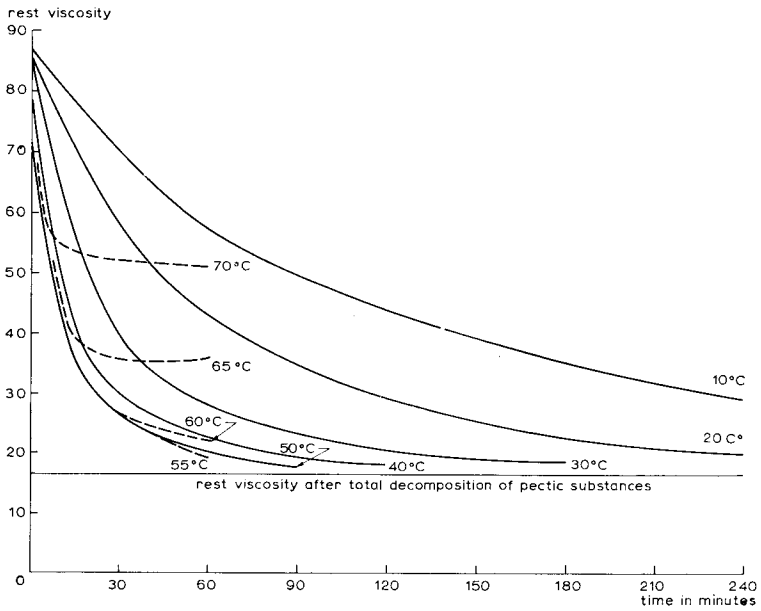


FIG. 54.
Influence of temperature upon breakdown of apple pectin by commercial pectolytic enzyme preparation. The viscosity of the solution is calculated as a percentage of original viscosity before enzyme addition (Krebs, 272).

degree of 'body'. In all cases the pectic enzymes have to be intimately mixed with the fruit pulp.

In Fig. 54 the relation between time and 'rest viscosity' (calculated as the percentage of the original viscosity) is plotted for a pectin containing apple juice to which a commercial enzyme preparation (Pectinol) has been added. Aliquots of this juice were held at different temperatures. At 70 °C and 65 °C the breakdown of pectic substances was not completed as a result of excessive inactivation of pectic enzymes.

The influence of heating of the freshly milled fruit pulps prior to enzyme treatment was discussed by HEIMANN ET AL (196) and CHARLEY (74).

HEIMANN ET AL (196) compared the results of various pre-treatments on pears, cherries, white and red grapes, black currants, raspberries and gooseberries. In all cases the best results (highest amount of soluble constituents in the juice) were obtained when the milled fruits had been heated (90 sec. at 80° C) prior to enzymic fermentation at 45° C. However, in most cases the beneficial effect of this heating prior to enzymic treatment was found to be rather slight. The soluble-solids content of the juices was also increased by an intensive breakdown of pectic substances before pressing.

CHARLEY (74) has pointed out that by heating of the freshly milled pulp to 80° C or higher, oxidative enzymic changes in the pulps are strongly decreased on account of the inactivation of oxidizing enzymes, but that innate pectinesterase is partially inactivated also. CHARLEY stressed that the presence of naturally occurring pectinesterase may be essential to promote the action of pectolytic enzyme preparations which show a pectinesterase deficiency. This point was exemplified in the case of black currants. Under-ripe fruits may be handled by an enzyme preparation with endo-polymethylgalacturonase (polygalacturonase Type II) or pectin trans-eliminase, since these fruits contain high-methoxyl pectins, but show a very low pectinesterase activity. On the other hand the pectic substances of over-ripe fruits are appreciably demethylated and are needing the action of endo-polygalacturonase, PG Type I (see p. 76).

In fruit juices and wines varying contents of methanol can be found, which must be attributed to the action of pectinesterase of the fruits or from enzyme preparations.

POLLARD and KIESER (383) have stated that, normally, pectic substances are not present in fermented ciders. The decomposition during fermentation has been found to be due to the action of a polygalacturonase of microbiological origin acting in conjunction with pectinesterase from the fruits. In the absence of pectinesterase, as in heat-treated juices, the high-ester pectins from the apples remain ungraded. According to BRAVERMAN and LIFSHITZ (61) pectinesterase is not produced during fermentation by *Saccharomyces cerevisiae*, but may be originating from other yeasts.

CRUESS, QUACCHIA and ERICSON (92) have claimed that use of pectic enzymes has proved to be very satisfactory in winemaking.

Inactivation of pectic enzymes in cloudy tomato and citrus juices

As reported before, inactivation of innate pectic enzymes is necessary for the stabilization of cloudiness in citrus and tomato juices.

The loss of cloud in these products has to be regarded as an important quality defect; the cloud is converted to a fluffy layer and separates from a clear supernatant liquid. Most of the flavour and colour compounds are adsorbed onto the solid particles in the juice and will be deposited with these particles when cloud stability is lost. Moreover, in citrus concentrates the pectic substances may form a jelly when they have been deesterified by pectinesterase.

When tomatoes are macerated at a relatively low temperature this 'cold-break' process results in a thin watery juice with a negligible content of pectic substances, which is inclined to show a loss of cloud during standing. It has been found that this breakdown of pectic substances takes place extremely rapid in a few minutes after maceration. This decomposition of pectic substances is caused by the polygalacturonase and pectinesterase which are present in the fruits (314). Even when the raw crushed tomatoes are rapidly heated, immediately after maceration, some breakdown of pectic substances is detectable (316).

The breakdown of pectic substances is prevented when a 'hot-break' process is applied by macerating the fruits at 82 °C or at a higher temperature. According to McCOLLOCH, NIELSEN and BEAVENS (315), for the manufacture of tomato juice the fruits have to be treated at 82 °C, while for tomato paste a heating to 85 °C is necessary. McCOLLOCH ET AL (315) have pointed out that these differences may be the result of operational differences between juice and paste processing. McCOLLOCH KELLER and BEAVENS (316) have stated that much of the activity of pectic enzymes in tomatoes can be destroyed by blanching the whole fruit in steam or hot water before crushing, since in these fruits (Pearson and San Marzano varieties) pectic enzymes occur, in highest concentration, near the surface of the fruit.

It should be stressed that the prevention of decomposition of pectic substances is not the only factor which governs the consistency of tomato juice or tomato paste.

LUH ET AL (293) have studied the relationship between ripeness level and consistency of the canned juice and found that juices made from soft-ripe tomatoes were thinner in consistency than juice from firm-ripe fruits. These differences have been partially explained by the higher protopectin content in firm-ripe tomatoes.

When the pulp was depectinized with versene and then recombined with the serum, the products showed a lower consistency. According to LUH ET AL (293) this fact is an indication that the pectic material in the tomato pulp particles is an important contributor to the consistency of the juice. However, the viscosity of the serum is important also. BAKER and GILLIGAN (25) reported the beneficial effect on cloud stability by the addition of polyphosphates, which may be expected to extract pectic substances from the pulp particles. Similar results have been obtained by DOESBURG (140) by addition of a small amount (0.05–0.10 %) of ammonium oxalate. It is to be expected that during heat treatments the extraction of pectins is promoted and that the swelling of pectic substances remaining in the pulp is increased by these additions.

Variations in consistency of the juice may be obtained also by using different finisher speeds. WHITTENBERGER and NUTTING (514) presented evidence that in general sheet-like or rod-like walls or wall fragments offer more resistance to flow and give a more stable juice structure than do spherical wall fragments. In later work (515) it was indicated that the viscosity of tomato juice is kept at a relatively low level by the presence of naturally occurring and added electrolytes and that the cellulose fraction should be regarded as the most important substance closely related to viscosity.

The consistency of tomato paste is influenced also by its content of pectic substances. Products with a low content (0.00–0.05 %) possess a poor quality, whereas a good quality showed to be related to a rather high (0.3–0.6 %) content (315). Since the pectic substances of tomato purees were found to be predominantly of low-methoxyl content (374), it is clear that the properties of the puree are influenced by the addition of calcium salts. The influence of addition of sodium hexametaphosphate (Calgon) and calcium chloride on the pectin content and serum viscosity of 'cold-break'

and 'hot-break' tomato puree was studied by PETERS ET AL (374). The viscosity of the serum from 'cold-break' samples (with a very low content of pectic substances) was not influenced by addition of calcium. Added increments of calcium to the serum of conventional 'hot-break' samples caused a slight increase of viscosity, followed by a marked decrease at the more concentrated calcium levels, presumably by further flocculation of the low-methoxyl pectic substances. When Calgon was added during maceration of 'hot-break' tomatoes, the serum of the finished tomato puree showed an increased viscosity at rather high calcium levels (100–200 ppm added).

The quality of citrus juices may be strongly influenced by the pectinesterase from the fruits. Though there is no reliable proof that depolymerizing enzymes occur in citrus fruits, in some cases slight evidence of their presence has been produced (246, 388).

As a result of pectinesterase activity, the pectic substances are saponified and precipitated by bivalent cations in the juice. The clarification, which occurs in unheated citrus juices is expected to be due mainly to pectinesterase activity. In citrus concentrates, which contain greater amounts of pectic substances, the saponified materials may form a jellied mass.

There is a complicated and voluminous literature on the precise conditions to prevent the action of pectinesterase in citrus products, which has been reviewed by PILNIK (376).

In contrast to tomato juices, the pectinesterase in citrus juices is attached completely to the pulp particles, thus pectinesterase activity is increased by an increased pulp content. However, in concentrates, the enzyme activity is not proportional to the degree of concentration. The highest activity is found in 4-fold concentrates (40° Brix), whereas at higher degrees of concentration the enzyme activity is strongly diminished by the increased soluble solids content as has been shown by MCCOLLOCH, RICE, GENTILLI and BEAVENS (317) (see p. 81).

For the production of citrus juices and concentrates most of the pectinesterase is inactivated by a heat treatment. The heat stability of the enzyme in citrus products has been studied by ATKINS ET AL (14, 15, 16, 17, 18), BISSET ET AL (49, 50, 51, 52), STEVENS ET AL (463), WENZEL ET AL (506) and PRATT and POWERS (388). Without holding time, heating to 96°C is necessary for complete inactivation of the in orange

TABLE 11. *Temperatures and holding times for sufficient cloud stabilization in juices with different pH (463).*

pH	Juice	Temperature	Holding time
3.8	orange	89°C	2 min.
		94°C	15 sec.
3.3	orange	85°C	2 min.
		90°C	15 sec.
3.2	grape-fruit	84°C	2 min.
		89°C	15 sec.
2.4	lemon	69°C	2 min.
		74°C	15 sec.

juices, whereas at temperatures above 90 °C a holding time of some seconds is needed (376).

According to STEVENS, PRITCHETT and BAIER (463) different temperatures and holding times are needed to effect satisfactory cloud stabilization in deaerated juices of average pulp content and with different pH.

As has been described for tomato products, the inactivation of the enzyme is not the only factor which is involved in cloud stabilization.

The stability of cloud is influenced also by the pressing operation and the finishing of the juice (412).

During the first steps of heating of orange juices, the enzyme activity is strongly diminished by the increasing temperatures, but during further elevation of temperature the effect on enzyme inactivation is smaller, whilst the effect on cloud stabilization is increasing. For this reason it is to be presumed that cloud stabilization is enhanced also by the effect of heating only (185, 376).

KIEFER (267) has stated that clarification of orange juices was intensified by addition of ascorbic acid. This effect could be counteracted by Fe^{2+} salts or KCN. For these reasons KIEFER concluded that, in addition to the enzymic effects, oxidation phenomena may be involved also.

The behaviour of juices during processing and storage life are influenced also by seasonal changes occurring in the pectinesterase activity and pectic constituents of the fruits, which have been investigated by ROUSE, ATKINS and MOORE (413, 414). Protopectin tended to decrease and the other components (water-soluble and oxalate-soluble pectic substances) tended to increase with maturity of the fruits. Pectinesterase activity showed some seasonal variation.

In the USA, frozen orange concentrates are manufactured by blending of pasteurized 6–fold concentrates with fresh juice to a 4–fold concentrate (42° Brix). The use of fresh unpasteurized juice gives a very high quality with a fresh character. However, as reported before the conditions for enzyme activity are known to be optimal in 4–fold concentrates. For this reason, it is necessary that the pectinesterase in the 6–fold concentrate is partially inactivated to prevent lumping or jelly formation in the final frozen product. This partial inactivation is usually done by heating to 70–80 °C, which causes about 75% inactivation (376). When the greater part of the enzyme activity is not destroyed, quality defects may be found during storage of the frozen concentrate at temperatures slightly higher than —18 °C.

The heat inactivation is often carried out between the first and second stage of the concentration process (376). However, more recently MOORE, ROUSE and ATKINS (339) made a two season study of the effect of heat treatment at intermediate stages of concentration of orange juices and showed it to be advisable, in some cases, for single-strength juice to be heat treated after extraction and finishing and before concentration.

Since small residual pectinesterase activities have been shown to cause cloud loss in several citrus products, and direct observation of cloud behaviour often takes too long for quality control, sensitive methods for the detection of enzyme activity are needed. PILNIK and ROTHSCHILD (379) showed that with their method as little as 0.5% residual enzyme activity can be reliably detected. MANHEIM and ZIV (298) concluded that the enzyme activity test is sufficiently sensitive to serve as an index of heat treatment. PRIMO YÚFERA ET AL (524, 525) noted the existance of a direct relation between the modification of pulp pectins and the concentrate gelling or the clarification of the rediluted juice; changes in pectic substances were measured by a specific histochemical colour reaction.

Raw materials for pectin production, apple pomace

The main sources for pectin production are citrus peel and apple pomace (apple marc), which are left over after the manufacture of citrus products and apple juice or cider.

Many other products contain pectic substances which are unsuitable for the manufacture of the common high-methoxyl pectins for use in pectin-sugar-acid jellies.

During the Second World War sugar-beet residues have been used in Germany; they are used also in Sweden. High-methoxyl pectins cannot be recovered from sugar-beet residues since the relatively low degree of esterification of the original pectic substances will be decreased further during the removal of acetyl groups which have to be split off for the optimal development of jellying power (see p. 31).

Sisal and flax (73) and sunflower heads (464) have been mentioned as other secondary sources for pectin production.

Citrus peels are shredded and heated to 95–98 °C to inactivate the pectinesterase (73). The peels can be preserved by dehydration.

Data on the amount and jellying power of pectic substances in citrus peels are rather scanty. KEFFORD (255) reviewed these data, which are mainly based upon the work of SINCLAIR and CRANDALL (433, 434, 435). Especially lemon peel and albedo showed to be rich sources. Citrus peels may contain 20–40 % pectic substances calculated on a dry basis (255). The content of the residues from Spanish citrus fruits after juice extraction was reported by FUERTES POLO and ROYO IRANZO (170). ROUSE and ATKINS (411) showed that during maturation the total pectin content in Pineapple oranges did not change greatly until it declined in late maturity.

For the manufacture of apple pectins mostly dry apple pomace is used. The use of dry pomace permits of apple pectin production throughout the year. Moreover dehydration of apple presscakes prevents disintegration during pectin extraction, which facilitates screening and filtering operations. However, the pectin yield may be reduced by dehydration.

KERTESZ (259) indicated that air temperatures of 80–90 °C or even higher can be used while the pomace is still wet, but that the air temperature must be lowered to 65–70 °C when a moisture content of 20–25 % is reached. However, KERTESZ pointed out that some manufacturers use finishing temperatures up to 82° C without a decrease of quality of the pomace. DRYDEN, WILLAMAN and HILLS (146) proved that apple pomace may be dried in a laboratory drier at 90 °C with maximum retention of grade. DOESBURG (140) found no harmful effect of heating of apple pomace with a moisture content of 12 % during two hours at 80 °C (temperature of pomace), whereas in the same time the quality was lowered by heating at 90 °C.

According to KERTESZ and GREEN (261), no mould growth could be observed on artificially infected apple pomace containing 20 % or less of moisture. Usually the pomace is dried to a moisture content of 4–8 %. CHARLEY (73) indicated that the moisture content of the pomace should not be higher than 12 %. The mean pectin content is then about 16 %, but variations have been found from 8–20 % (366). JOHAR, KRISHNAMURTHY and BATHIA (236) found a mean content of nearly 17 %.

According to DRYDEN ET AL (146), the grade of a pomace may be defined as the

number of grams of sugar that can be converted into a jelly of standard strength by the pectin extracted from one gram of dry pomace.

For the evaluation of the grade value of pomace and citrus peels the product has to be extracted. A modification of the method of MOTTERN and KARR (342) was used by DRYDEN ET AL (146). In practice, several other methods are used, which are not described in the literature.

In the method described by DRYDEN ET AL (146), 100 g of ground pomace (previously ground in a Wiley mill to pass a 4-mm screen) are weighed into a tared 2 liter beaker, then four g of sodium tetraphosphate, $Na_6P_4O_{13}$, and 1500 ml water are added and the pH of the mixture is adjusted to 3.2 by the addition of hydrochloric acid. The mixture is then heated to the boiling point and held at 90–100°C for 30 minutes. After cooling to 50°C, the beaker is weighed and net contents recorded. After draining of the suspension through cheesecloth suitable amounts of extracts are taken for the preparation of test jellies. From the jellying power of the extract, the jelly grade of pomace can be calculated.

Extraction of 200 g pomace (without previous grinding) and extraction during two hours at pH 2.0 $\pm$ 0.1 at 80–85°C is performed in the method of DOESBURG (140). The pomace is weighed in 4-liter beakers; after addition of 3200 g water the suspension is acidulated with hydrochloric acid to pH 1.8, which yields a final pH $\pm$ 2.0 during extraction. The extraction is carried out by placing the beaker in a boiling waterbath (Fig. 55); the temperature of the waterbath is lowered to 85°C when the pomace suspension has been heated to 80°C and then the extraction is continued for two hours. During heating and extraction the suspension is slowly mechanically stirred (30 rpm). Thereafter the weight of the suspension is adjusted to 3400 g and drained through cheesecloth. A part of the filtrate is cooled to $\pm$ 15°C and is then mixed rapidly with some strong NH_3-solution to adjust to pH 3.5, prior to the performance of jelly tests. This evaluation of pomace grades has shown to correspond well with the results of commercial pectin manufacture.

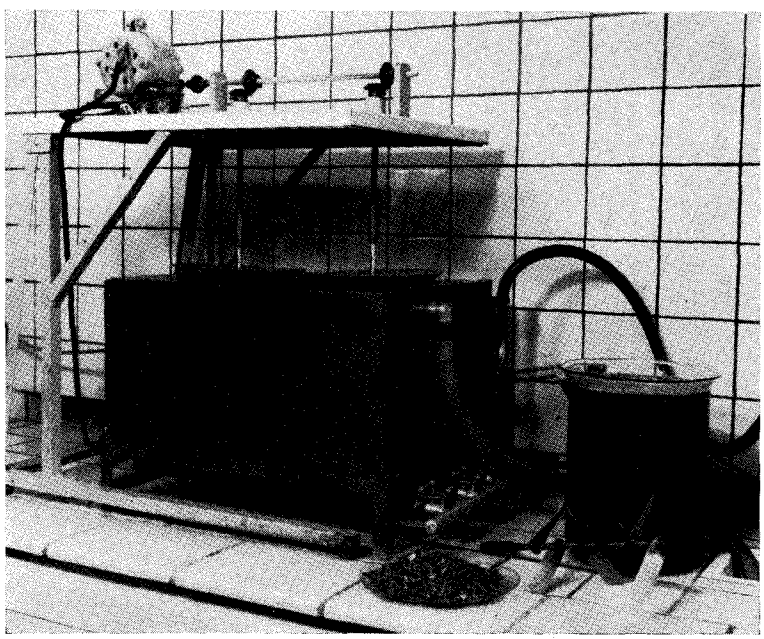


FIG. 55.
Equipment for extraction of apple pomace in order to determine the jelly grade of pomace (Doesburg, 140).

The jelly grade of apple pomace may be affected by several factors. When the pomace has been made from rather ripe fruits an important part of the total amount of pectic substances is removed during pressing of the juice, since $\pm$ 40% of the pectic substances in apples are solubilized during ripening. DRYDEN ET AL (146) investigated the grades of pomace manufactured by an American commercial apple juice plant from October to March. The pomace grades decreased from the beginning of October to December and then increased again. The first decrease is caused by solubilization of pectins during ripening of fruits in common stores, whereas the increase of grade of pomace manufactured from December to March was related to use of apples from cold storage, which ripened very slowly. From practical experience in the Netherlands, it was noted several times that apples from cold stores yielded a low-grade pomace when pressed in February.

As shown by DOESBURG (126) the quality of pomace is influenced also by the variety from which it is produced. Little is known about the influence of growing conditions of the fruits. In Fig. 56 the influence of maturity and variety is shown when the pomace is manufactured from freshly picked apples, harvested at different stages of maturity. It can be seen that pomace quality shows a great variation which is influenced by the variety and the maturity stage of fruits. BURROUGHS ET AL (68) investigated the influence of maturity and found also a decrease of pomace quality during ripening, but this decrease was less pronounced as shown for several varieties in Fig. 56.

The jellying power of apple pomace from fruits of different varieties and maturity stage seemed to depend only on different pectin contents. The jellying power of pure pectins (calculated from titrimetrical determinations and Wageningen jelly tests) proved to be 500–550 Wageningen grades (125), which corresponds with 300–340 I.F.T.-jelly grades (228). Similar jellying power of pure pectins has been noted for pure citrus pectin preparations.

As reported earlier, apple pectins are not degraded during ripening (see p. 92). Soluble pectins have about the same jelly grade as the insoluble pectic substances after extraction. This is in contrast with other opinions (259) which indicate that only degraded soluble pectins are removed during leaching in cold water for removal of coloured materials prior to extraction for pectin manufacture. MOTTERN and HILLS (343) found that on leaching by water at 50°C a considerable amount of pectins is lost. DESCHREIDER (104) has stated that various pomace samples show a different sensitiviness to leaching with water; in all cases, pectin losses should be strongly limited by the use of water at 20°C or a lower temperature.

DOESBURG (126) has investigated the quality of pomace produced from a great number of apple varieties at different stages of maturity. Within the whole range, no correlation existed between development of colour (green to yellow) of fresh fruits and the quality of pomace manufactured from them, but there was a distinct positive correlation ($r = + 0.69$, $n = 53$, $P < 0{,}001$) between the results of pressure tests on fresh fruits and pomace quality.

DRYDEN ET AL (146) compared the jelly grades of pressed and dehydrated pomace of peels and cores and of the pressed and dehydrated remainder of the fruits. The pomace from peels and cores was from 25 to 70% higher in grade than that from the rest of the apple tissue.

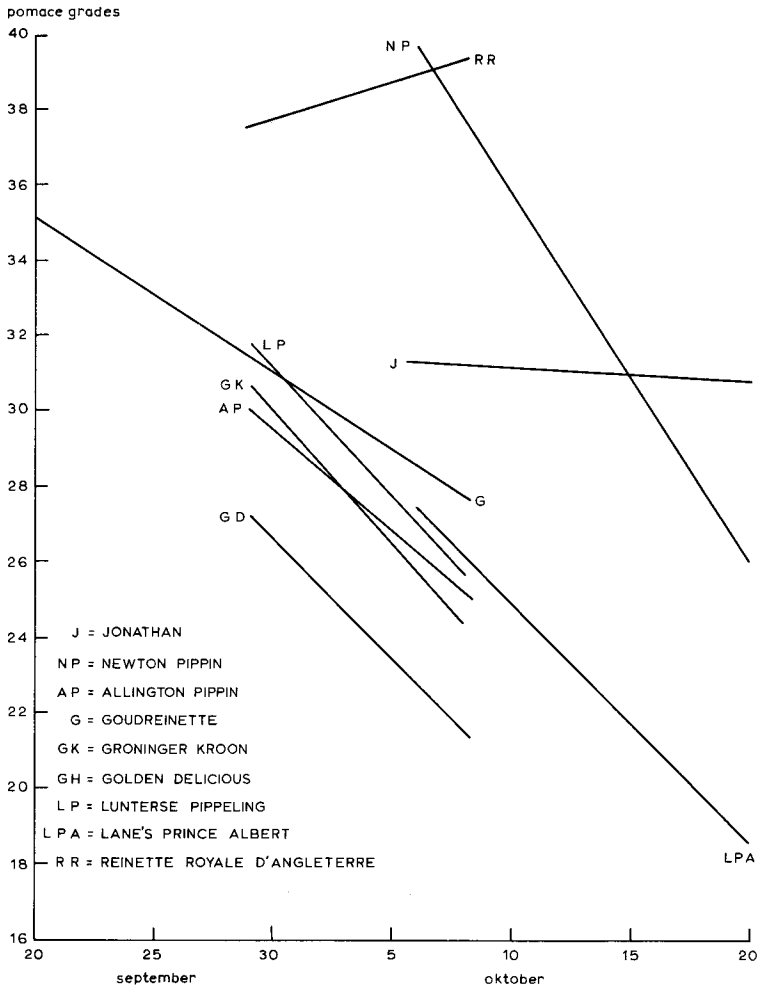


FIG. 56. Influence of degree of maturity of apples of different varieties on the jelly grade of pomace (Doesburg, 126).

The results of the analysis of pectin content, degree of esterification and jellying power of fresh peels, cores and the middle parts of apples from three varieties in January are shown in Table 12.

In all cases the amount of pectins extracted from the peels was highest, whilst the content of cores is greater than that of the middle part. The distribution of jellying power showed the same trend; in two cases the jellying power of pure pectins from the cores proved to be slightly lower than from the other parts. In all varieties, the degree of esterification of pectins from the cores was lower also.

According to the experiences of the author, pomace from peels and cores swells very strongly during extraction. To establish suitable conditions for extraction of dehydrated peels and cores it showed to be necessary to use twice the amount of water needed for extraction of the same amount of dehydrated pomace which is prepared after milling and pressing of the complete fruits.

It is necessary for the presscakes to be preserved within a very short time after pressing, since the yield of jellying units is decreased by standing of the wet product (moisture content $\pm$ 65%).

TABLE 12. *Distribution of pectin contents and jellying power (Wageningen jelly grades) over several parts of apple fruits, January 1950 (140).*

Variety	Part of fruits	Jelly grades of tissue	Pectin content	Degree of esterification	Jelly grade of pure pectin
Bellefleur	peel	3.63	0.67	75	543
	middle	1.85	0.34	75	543
	core	2.49	0.45	63	533
Belle de Boskoop	peel	2.99	0.57	75	522
	middle	1.19	0.24	76	501
	core	1.62	0.33	69	480
Bramley's Seedling	peel	2.42	0.47	73	510
	middle	1.08	0.21	70	519
	core	1.30	0.29	66	450

It has been proved that the pectin quality of wet presscakes can be preserved temporarily by addition of sulphur dioxide. When the pomace is not dehydrated for some time after a previous SO_2 treatment, the product has to be heated to inactivate a weak enzyme activity. These small amounts of enzymes have been produced already by moulds on the fruits or from rotten parts of fruits before the moment of pressing (see p. 97). CHARLEY ET AL (75) and BURROUGHS ET AL (68) indicated that prior to dehydration the wet pulp may be preserved temporarily (one or two days) by 250–500 ppm sulphur dioxide. SERGER (429) advised the addition of 2.0–2.5 l 6%SO_2 solution to preserve wet pomace packed in barrels.

CHARLEY, BURROUGHS, KIESER and STEEDMAN (75) found 21–39% losses after one day and losses of 40–44% after two days storage of the non-preserved wet pulp. DRYDEN ET AL (146) noted a loss of 40% after one day and about 50% after two days. Within one day a faint mouldy odour was detectable and after three days this odour was quite strong and mould growth distinctly visible. SULČ and RISTIČ (469) proved that partial depolymerization of pectic substances occurs without an accompanying loss of pectic substances. For this reason SULČ and RISTIČ advise that the wet pulp should be preserved by dehydration within 2–3 hours after pressing.

Since innate depolymerizing enzymes have not been found in apples and these fruits are showing only a very weak pectinesterase activity, it is presumed that the breakdown of pectic substances is caused by enzymes produced by microbial contamination.

Though the prevention of microbial attack of wet presscakes is important, it has to be stressed that other factors may also be involved. DEUEL (107) has indicated already that a part of the breakdown of pectic materials in fruit pulps might be related to oxidative decomposition of ascorbic acid. The work of JOSLYN and DEUEL (250) shows that enzymic browning of apple tissue markedly decreases the solubility of pectins, but does not alter the composition of pectins extracted. The solubility of pectins in fresh or dried commercial apple pomace was markedly lower than that of carefully prepared non-oxidized apple tissue. The lower extractibility of pectins from oxidized pomace may be due to the formation of polymerized oxidation products of the phenolic substrates in the apple tissue, which would decrease diffusion of solvent into the tissue or the outward diffusion of solubilized pectins. It could not be shown that pectins are made insoluble by chemical reactions with the formed

oxidation products. For this reason, it has to be supposed that their extractability is hindered by mechanical enmeshing of pectins by polymerized oxidation products (see p. 17). According to PILNIK (377a), the extent of desintegration of citrus peel during extraction is proportional to the pectin yield, which may be related to the absence of enzymatic browning in citrus fruits (60a).

JOSLYN and DEUEL (250) proved that pre-treatment of apple marc preparations with SO_2 increased the yield of pomace preparations. However, it has to be remembered that oxidative browning is inhibited also by sulphur dioxide. For these reasons it has to be expected that the beneficial effect of addition of sulphur dioxide to wet pomace results from its prevention of enzymic oxidation and the inhibition of microbial growth.

It has been reported already that DOESBURG (126) investigated the jelly grades of pomace produced from fruits of a great number of apple varieties and with a varying degree of maturity. The brown colour of the dried pomace was measured also. He showed that, over the whole range, a reliable negative correlation could be found between the jelly grade and intensity of brown colour of the dried pomace samples ($r = -0.52$, $n = 53$, $P < 0.001$), which is another indication that discoloration may be a factor which decreases the extractability of pectins. Therefore the extractability of pectins and the disintegration of pomace during extraction may be decreased when the pomace is manufactured from apples with a high content of polyphenolic substances.

DOESBURG (123, 124) showed that oxalates are powerful inhibitors of enzymic brown discolorations, especially in apples. The browning of milled apples can be checked completely by the addition of 0.05–0.10 % ammonium oxalate, since the polyphenol oxidase is inhibited (124).

The influence of addition of ammonium oxalate was studied by milling of two identical lots of Belle de Boskoop apples, which develop a rather intensive enzymic browning after being milled. In the first lot, the browning was checked by addition of 0.05 % ammonium oxalate during milling; to the other lot nothing was added (140). After milling, both lots were pressed and stored and after various storage times an aliquot of each lot was preserved by dehydration. Finally, after some weeks, the dehydrated aliquots were extracted. During extraction (2 hours at 85 °C; pH 2.0) an amount of ammonium oxalate, corresponding with the content of the first lot, was also added to the pomace made from the aliquots of the untreated lot in order to perform the extraction under comparable conditions. The results of these trials are shown in Table 13.

TABLE 13. *Influence of addition of ammonium oxalate during milling of apples upon the jelly grades of pomace, dehydrated at different times after milling (140).*

storage time of wet pulp prior to dehydration	jelly grades of dehydrated pomace		jelly grades of extracted pure pectins		degree of esterification %	
	oxalate added	no oxalate added	oxalate added	no oxalate added	oxalate added	no oxalate added
1 h	15.0	15.2	510	500	75	76
4 h	15.5	13.7	517	521	76	74
6 h	13.3	—	494	—	74	—
7 h	—	8.7	—	490	—	74
23 h	14.5	5.8	502	507	75	76

It is clear that, in this experiment, prevention of enzymic browning of wet pulp by oxalates has improved the stability of jelly grades prior to dehydration. Nevertheless there was only a slight colour difference between dehydrated, oxalate-treated, samples and dehydrated, non-treated samples. The nature of colour compounds in the dehydrated pomace was found to depend on the pre-treatment

of the wet pomace. The dehydrated pomace of oxalate-treated apple marc contained a red-brown colour which was solubilized during pectin extraction, whereas the untreated pomace has a less-extractable brown colour. For this reason the extracts from oxalate-treated pomace were more strongly coloured than the extracts from pomace of fruits which had been milled without oxalate addition; however, in the oxalate-treated dehydrated pomace the extraction of pectin was not hindered by these soluble coloured materials. DOESBURG (123, 124) reported that in other products also the nature of discolouration was influenced by oxalate additions.

Finally some remarks have to be made on the possible influence of non-enzymic browning in dehydrated pomace. It is known that such reactions cause the formation of polymer compounds, which may also be expected to decrease extractability of pectins. The rate of this reaction type depends on the moisture content of the products during storage. In this type of products, the maximum rate occurs at a moisture content of 10–20%. Further, such reactions show a rather high temperature coefficient (Q_{10} about 6–8). Thus, the decrease of pectin quality during storage can be limited by a rather low moisture content (4–8%) and a low storage temperature (259, 261). Further it has to be remembered that the reaction is inhibited also by the presence of sulphur dioxide.

Literature

A

1 AHMED, E. and L. E. SCOTT, Proc. Am. Soc. Hort. Sci. 71 (1958) 376.
2 ALBERSHEIM, P., Biochem. Biophys. Res. Commun. 1 (1959) 253.
3 ALBERSHEIM, P., Plant Phys. 36 (1961) suppl. p. XXXIX.
4 ALBERSHEIM, P., H. NEUKOM and H. DEUEL, Arch. Biochem. Biophys. 90 (1960) 46.
5 ALBERSHEIM, P., H. NEUKOM and H. DEUEL, Helv. Chim. Acta 43 (1960) 1422.
6 ALBERSHEIM, P. and U. KILLIAS, Arch. Biochem. Biophys. 97 (1962) 107.
7 ANDERSON, D. B., Bot. Rev. 1 (1935) 52.
8 ANTHISTLE, M. J., Fruit and Veg. Cann. and Quick Freezing Res. Assoc., Chipping Campden. Engl., Sci. Bull. No. 4, (1961).
9 ANYAS-WEISZ, L., Experientia, 9–2 (1953) 64.
10 ANYAS-WEISZ, L., L. SOLMS and H. DEUEL, Mitt. Geb. Lebensm. Unters. Hyg. 42 (1951) 91.
11 ARCHER, R. W., Canner and Packer, 131 (1962, no. 9) 28.
12 ASH, A. S. F. and T. M. REYNOLDS, Austr. J. Biol. Sci. 7 (1954) 435.
13 ASPINALL, G. O., Ann. Rev. Biochem. 31 (1962) 79.
14 ATKINS, C. D., R. L. HUGGART and F. W. WENZEL, Proc. Florida State Hort. Soc. (1952) 217.
15 ATKINS, C. D., A. H. ROUSE, R. L. HUGGART, E. L. MOORE and F. W. WENZEL, Food Technol. 7 (1953) 62.
16 ATKINS, C. D. and A. H. ROUSE, Food Technol. 7 (1953) 489.
17 ATKINS, C. D. and A. H. ROUSE, Food Technol. 8 (1954) 498.
18 ATKINS, C. D., A. H. ROUSE and E. L. MOORE, Proc. Florida State Hort. Soc. (1956) 181.
19 AYRES, A., J. DINGLE, A. PHIPPS, W. W. REID and G. L. SOLOMONS, Nature 170 (1952) 834.

B

20 BAIER, W. E., and C. W. WILSON, Ind. Eng. Chem. 33 (1941) 287.
21 BAKER, G. L., Ind. Eng. Chem. 18 (1926) 89.
22 BAKER, G. L. and M. W. GOODWIN, Delaware Agr. Expt. Sta., Bull. 234, Techn. No. 28 (1941).
23 BAKER, G. L. and M. W. GOODWIN, Delaware Agr. Exp. Sta., Bull. 246, Techn. No. 31 (1944).
24 BAKER, G. L. and C. W. WOODMANSEE, Fruit Prod. J. 23 (1944) 164.
25 BAKER, G. L. and G. M. GILLIGAN, Fruit Prod. J. 26 (1947) 260.
26 BAKER, G. L. and W. G. MURRAY, Food Research 12 (1947) 129.
27 BAKER, G. L. and C. W. WOODMANSEE, Delaware Agr. Expt. Sta. Bull. 272, Techn. No. 40 (1948).
28 BAKER, G. L. and C. W. WOODMANSEE, Food Technol. 3 (1949) 23.
29 BAKER, G. L., J. F. KULP and R. A. MILLER, Food Research 17 (1952) 36.
30 BALATSOURAS, G. D. and R. H. VAUGHN, Food Research 23 (1958) 235.
31 BATE-SMITH, E. C., Qual. plant. mater. veg. 3/4 (1958) 440.
32 BEAVEN, G. H., E. L. HIRST and J. K. N. JONES, J. Chem. Soc. (1939–2) 1865.
33 BEAVEN, G. H. and F. BROWN, Bioch. J. 45 (1949) 221.
34 BELL, T. A., Bot. Gaz. 113 (1951) 216.
35 BELL, T. A., J. L. ETCHELLS and I. D. JONES, Food Technol. 4 (1950) 157.
36 BELL, T. A., J. L. ETCHELLS and I. D. JONES, Arch. Biochem. 31 (1951) 431.
37 BELL, T. A., J. L. ETCHELLS and I. D. JONES, U. S. Dept. Agric., Agric. Res. Serv. A.R.S. 72–5 (1955).
38 BELL, T. A. and J. L. ETCHELLS, Bot. Gaz. 119 (1958) 192.
39 BELL, T. A., A. W. AURAND and J. L. ETCHELLS, Bot. Gaz. 122 (1960) 143.
40 BELL, T. A. and J. L. ETCHELLS, J. Food Sci. 26 (1961) 84.

41 BELL, T. A., J. L. ETCHELLS, C. F. WILLIAMS and W. L. PORTER, Bot. Gaz. 123 (1962) 220.
41a BELL, T. A., J. L. ETCHELLS, J. A. SINGLETON and W. W. G. SMART, J, Food Sci. 30 (1965) 233.
42 BENEKE, E. S., L. S. WHITE and F. W. FABIAN, Appl. Microbiol. 2 (1954) 253.
43 BENNISON, E. W. and F. W. NORRIS, Bioch. J., 33 (1939–II) 1443.
44 BETTELHEIM, F. A. and C. STERLING, Food Research 20 (1955) 71.
45 BETTELHEIM, F. A. and C. Sterling, Food Research 20 (1955) 118.
46 BETTELHEIM, F. A. and C. STERLING, J. Polym. Sci. 22 (1956) 303.
47 BILIMORIA, M. and J. V. BHAT, J. Indian Inst. Sci. 44 (1962) 15.
48 BISHOP, C. T., Can. J. Chem. 33 (1955) 1521.
49 BISSET, O. W., M. K. VELDHUIS and N. B. RUSHING, Food Technol. 7 (1953) 260.
50 BISSET, O. W., M. K. VELDHUIS and N. B. RUSHING, Food Technol. 8 (1954) 38.
51 BISSET, O. W. and M. K. VELDHUIS, Proc. Florida State Hort. Soc. (1956) 109.
52 BISSET, O. W., M. K. VELDHUIS, R. B. GUYER and W. M. MILLER, Food Technol. 11 (1957) 512
53 BLOOM, O. T., U.S. Pat. 1, 540, 979 (1925).
54 BOCK, H., Theory and practice of pectin production, Board of Trade, German Division (Documents Unit), London (1946).
55 BOCK, W. and D. LANGE, Nahrung 7 (1963) 71.
56 BOGGS, M. M., H. CAMPBELL and C. D. SCHWARTZE, Food Research 7 (1942) 272.
57 BOGGS, M. M. and G. JOHNSON, Food Inds 19 (1947) 1067.
58 BOREL, E., Ueber die quantitative Zuckerbestimmung mit 3,4-dinitrobenzoësäure, Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1953).
59 BOYLE, F. P., Z. I. KERTESZ, R. E. GLEGG and M. A. CONNOR, Food Research 22 (1957) 89.
60 BRACONNOT, H., Ann. chim. et phys. Ser. 2, 28 (1825) 173.
60a BRAVERMAN, J. B. S., Introduction to the biochemistry of foods, Elseviers Publishing Cy, Amsterdam–London–New York (1963).
61 BRAVERMAN, J. B. S. and A. LIFSHITZ, Food Technol. 11 (1957) 356.
62 BREKKE, J. E. and G. C. WATTERS, Canner and Packer 132 (1963, no. 8) 29.
63 BRINTON, C. S., H. J. WICHMANN, J. J. WILLAMAN, C. P. WILSON and W. H. DORE, Proc. Am. Chem. Soc. 49 (1927) 38.
64 BROOKS, J. and W. W. REID, Chem. & Ind. (1955) 325.
65 BUCH, M. L., K. G. SATORI and C. H. HILLS, Food Technol. 15 (1961) 526.
66 BÜCHI, W. Ueber ein Polysaccharid in Beerensaft von Vitis vinifera L., Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1954).
67 BÜCHI, W. and H. DEUEL, Helv. Chim. Acta 37 (1954) 1392.
68 BURROUGHS, L. F., M. E. KIESER, A. POLLARD and J. STEEDMAN, Ann. Rep. Agr. Hort. Res. Sta. Long Ashton (1943) 136; Fruit Prod. J. 24 (1944) 4.

C

69 CAMPBELL, L. E., J. Soc. Chem. Ind. 57 (1938) 413.
70 CARRÉ, M. H. and D. HAYNES, Biochem. J. 16 (1922) 60.
71 CARTER, G. H., D. W. INGALSBE, A. M. NEUBERT and E. L. PROEBSTING, Food Technol. 12 (1958) 174.
72 CASS, W. G., Food 16 (1947) 332.
72a CHANG, L. W. S., L. L. MORITA and H. Y. YAMAMOTO, J. Food Sci. 30 (1965) 218.
73 CHARLEY, V. L. S., Chem. & Ind. (1951) 394.
74 CHARLEY, V. L. S., in: Production and application of enzyme preparations in food manufacture, S.C.I. Monograph No. 11, Soc. Chem. Ind., London (1961) 107.
75 CHARLEY, V. L. S., L. F. BURROUGHS, M. E. KIESER and J. STEEDMAN, Ann. Rep. Agr. Hort. Res. Sta., Long Ashton (1942) 89.
76 CHEFTEL, H., Utilisation industrielle des fruits, Etabl. J. J. Carnaud et Forges de Basse-Indre, Paris, Bull. nr. 7 (1948).
77 CHEFTEL, H. and J. MOCQUARD, J. Soc. Chem. Ind. 66 (1947) 297.
78 CHRISTENSEN, P. E., Food Research 19 (1954) 163.
79 CLARK, E. P., J. Am. Chem. Soc. 51 (1929) 1480.

80 CLARK, E. P., J. Offic. Agr. Chem. 15 (1932) 136.
81 CLARKE, I. D., Intern. Congr. Food Sci. Technol. London (1962); Abstr. Fruchtsaft Ind. 3 (1963) 180.
82 CLAYPOOL, L. L., Suggested use of the pressure tester for canning pears, circ. Dept. Pomol., Univ. California.
83 COLE, G. M., R. E. COX and G. H. JOSEPH, Food Inds. 2 (1930).
84 COLIN, H. and A. CHAUDUN, C. r. Acad. Sci. Paris 201 (1935) 407.
85 COLIN, H. and A. CHAUDUN, C. r. Acad. Sci. Paris 202 (1936) 973.
86 COLLINS, J. L. and R. C. WILEY, Influence of added calcium salts on texture of thermal-processed apple slices, Univ. Maryland, Agr. Exp. Sta. (1963).
87 CORVAISIER, H. LE, Chim. et Ind. 57 (1947) 545.
88 COX, R. E. and R. H. HIGBY, Food Inds 16 (1944) 441.
89 CRAFTS, A. S., Food Inds 16 (1944) 184.
90 CREAN, D. E. C. and D. R. Haisman, J. Sci. Food Agric. 14 (1963) 824.
91 CRUESS, W. V., Fruit Prod. J. 27 (1947) 44.
92 CRUESS, W. V., R. QUACCHIA and K. ERICSON, Food Technol. 9 (1955) 601.

D

93 DAKIN, J. C., Nature 199 (1963) 383.
94 DAME, C., S. J. LEONARD, B. S. LUH and G. L. MARSH, Food Technol. 10 (1956) 28.
95 DATE, W. B. and E. HANSEN, Proc. Indian Acad. Sci. 39, section B (1954) 171.
96 DAVIGNON, L., Fruits, 15 (1960) 469.
97 DAVIGNON, L., Annexe Bull. Intern. Inst. Refrigeration (1961–1) 24.
98 DAVIGNON, L., Ph-D. Thesis University of Paris (1961).
99 DAVISON, F. R. and J. J. WILLAMAN, Bot. Gaz. 83 (1927) 329.
100 DEMAIN, A. L. and H. J. PHAFF, J. biol. Chem. 210 (1954) 381.
101 DEMAIN, A. L. and H. J. PHAFF, Nature 174 (1954) 515.
102 DEMAIN, A. L. and H. J. PHAFF, J. Agr. Food Chem. 5 (1957) 61.
103 DEMAIN, A. L. and H. J. PHAFF, Wallerstein Labs Commun. 20 (1957) 119.
104 DESCHREIDER, A. R., Revue Ferment. et Ind. Alim. 3 (1948) 14.
105 DESCHREIDER, A. R. and S. VAN DEN DRIESSCHE, Food Manufact. (1948) 77.
106 DEUEL, H., Ber. Schweiz. Bot. Ges. 53 (1943) 219.
107 DEUEL, H., Helv. Chim. Acta 26 (1943) 2002.
108 DEUEL, H., Mitt. Lebensmitt. unters. Hyg. 34 (1943) 41.
109 DEUEL, H. and F. WEBER, Helv. Chim. Acta 28 (1945) 1089.
110 DEUEL, H. and H. NEUKOM, J. Polym. Sci. 4 (1949) 759.
111 DEUEL, H. and G. HUBER, Helv. Chim. Acta 33 (1950) 10.
112 DEUEL, H., G. HUBER and L. ANYAS-WEISZ, Helv. Chim. Acta 33 (1950) 563.
113 DEUEL, H. and W. EGGENBERGER, Kolloid-Zschr. 117 (1950) 97.
114 DEUEL, H. and J. SOLMS, Kolloid-Zschr. 124 (1951) 65.
115 DEUEL, H., J. SOLMS and A. DENZLER, Helv. Chim. Acta 36 (1953) 1671.
116 DEUEL, H., J. SOLMS and H. ALTERMATT, Vierteljahrsschrift Naturforsch. Ges. Zürich 98 (1953) 49.
117 DEUEL, H., J. SOLMS and A. DENZLER, Mitt. Geb. Lebensmitt. unters. Hyg. 45 (1954) 73.
118 DEUEL, H., K. HUTSCHNEKER, E. STUTZ and J. C. FREDERIKS, Helv. Chim. Acta 40 (1957) 2009.
119 DEUEL, H. and E. STUTZ, Adv. Enzymol. 20 (1958) 341.
120 DICKINSON, D., J. Sci. Food Agric. 10 (1959) 73.
121 DICKINSON, D. and C. L. HINTON, Fruit and Veg. Canning and Quick Freezing Res. Assoc., Chipping Campden, Techn. Bull. No. 3 (1955).
122 DISCHE, Z., J. biol. Chem. 183 (1950) 489.
123 DOESBURG, J. J., Meded. Dir. Tuinb. 12 (1949) 82.
124 DOESBURG, J. J., Meded. Dir. Tuinb. 12 (1949) 150.
125 DOESBURG, J. J., Voeding 11 (1950) 138; Food Sci. Abstr. 25 (1953) 328.
126 DOESBURG, J. J., Meded. Dir. Tuinb. 14 (1951) 609.
127 DOESBURG, J. J., Proc. VIIIth Intern. Congr. Refrig., London (1951) 548.

128 DOESBURG, J. J., Annexe Bull. Intern. Inst. Refrigeration (1954–1) 59.
129 DOESBURG, J. J., Voeding 16 (1955) 503.
130 DOESBURG, J. J., Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, Rep. No. 798 (1957).
131 DOESBURG, J. J., J. Sci. Food Agr. 8 (1957) 206.
132 DOESBURG, J. J., Conserva 7 (1958/59) 276.
133 DOESBURG, J. J., Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, Rep. No. 715 (1959).
134 DOESBURG, J. J., Ann. Rep. Inst. Res. Stor. & Proc. Hort. Prod. Wageningen, Netherlands (1960), 66.
135 DOESBURG, J. J., Ind. Obst u. Gemüseverwert. 45 (1960) 51.
136 DOESBURG, J. J., Annexe Bull. Intern. Inst. Refrigeration (1961–1) 29.
137 DOESBURG, J. J., Qual. plant. mat. vegetab. 8 (1961) 115.
138 DOESBURG, J. J., Conserva 11 (1962/63) 8, 29.
139 DOESBURG, J. J., Conserva 12 (1963/64) 107.
140 DOESBURG, J. J., unpublished results.
141 DOESBURG, J. J. and G. GREVERS, Conserva 1 (1952/53) 150; Food Sci. Abstr. 27 (1952) No. 871.
142 DOESBURG, J. J. and L. DE VOS, V Intern. Fruit Juice Congr., Vienna (1959) 32.
143 DOESBURG, J. J. and G. GREVERS, Food Research 25 (1960) 634.
144 DOESBURG, J. J. and L. DE VOS, Fruits 15 (1960, no. 8).
145 DREWES, K., Molkerei Zeit. 49 (1935) 421.
146 DRYDEN, E. C., J. J. WILLAMAN and C. H. HILLS, Food Technol. 6 (1952) 474.
147 DUDEN, R., Fruchtsaft Ind. 9 (1964) 69.
148 DURACH, W., Ind. Obst. u. Gemüseverwert. 37 (1952) 417.
149 DUROCHER, J. and G. ROSKIS, Officiel de la Conserve 4 (1949) 25.
150 DZÁMIC, M., Rev. Res. Work. Fac. Agr. Belgrade 2 (1954) 213.

E

151 EDWARDS, J. W. and M. A. JOSLYN, Arch. Biochem. Biophys. 39 (1952) 51.
152 EGGENBERGER, W., Biochemische Untersuchungen an Apfeln während der Entwickelung und Lagerung, Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1949).
153 EHRLICH, F. and R. V. SOMMERFELD, Biochem. Zschr. 168 (1926) 263.
154 ELLWELL, W. E. and W. M. DEHN, Plant Physiol. 14 (1939) 809.
155 EMMETT, A. M., Biochem. J. 20 (1926) 564.
156 ENDO, A., Agric. biol. Chem. 25 (1961) 394.
157 ENDO, A. and Y. MIURA, Agric. biol. Chem. 25 (1961) 382.
158 ESAU, P., M. A. JOSLYN and L. L. CLAYPOOL, J. Food Sci. 27 (1962) 509.
159 ETCHELLS, J. L., T. A. BELL and I. D. JONES, Food Technol. 9 (1955) 14.
160 ETCHELLS, J. L., T. A. BELL and C. F. WILLIAMS, Food Technol. 12 (1958) 204.
161 ETCHELLS, J. L., T. A. BELL, R. J. MONROE, P. M. MASLEY and A. L. DEMAIN, Appl. Microbiol. 6 (1958) 427.

F

162 FELLERS, C. R. and J. A. CLAGUE, Ind. Eng. Chem., Anal. Ed. 4 (1932) 106.
163 FENNEMA, O. and K. G. WECKEL, Univ. Wisconsin, Research Bull. 224 (1961).
164 FODA, Y. H., Ph-D. Thesis Univ. Illinois (1957); Diss. Abstr. 17 (1957) 2405.
165 FOWLER, H. D., J. Sci. Food Agric. 7 (1956) 381.
166 FOWLER, H. D., J. Sci. Food Agric. 8 (1957) 333.
167 FREEMAN, M. E. and W. S. RITCHIE, Food Research 5 (1940) 167.
168 FREY-WYSSLING A., Protoplasma 25 (1936) 261.
169 FREY-WYSSLING, A., Submicroscopic morphology of protoplasm, 2nd. Edition, Elsevier Publishing Co., Amsterdam (1953).
170 FUERTES POLO, C. and J. ROYO IRANZO, Ion 14 (1954) 455.

G

171 GALSTON, A. W., The life of the green plant. Prentice Hall, Inc., Englewood Cliffs, (N.Y.). 1961.
172 GANE, R. and H. G. WAGER, in: Plant structure and dehydration, S. C. I. Monograph no. 3, Soc. Chem. Ind., London (1958).
173 GEE, M. and R. M. MCCREADY, Food Research 22 (1957) 300.
174 GEE, M., R. M. REEVE and R. M. MCCREADY, J. Agr. Food Chem. 7 (1959) 34.
175 GELDERMALSEN-DE JONGH, E. VAN, personal communication (1962).
176 GERSONS, L., personal communication (1964).
177 GERSONS, L., J. J. DOESBURG and H. G. SCHURINK, Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, Report no. 522 (1954).
178 GERSONS, L. and J. J. DOESBURG, Inst. Res. Stor. & Proc. Hort. Prod. Wageningen Netherlands, Report no. 745 (1957).
179 GERSONS, L. and M. P. KARELSE, Conserva 6 (1957/58) 281.
180 GLEGG, R. E. and Z. I. KERTESZ, Science 124 (1956) 893.
181 GLEGG, R. E., T. P. BOYLE, L. W. TUTTLE, D. E. WILSON and Z. I. KERTESZ, Radiation Res. 5 (1956) 127.
182 GRIEBEL, C. and F. WEISS, Zschr. Unters. Lebensm. 58 (1929) 189.
183 GRIFFIN, J. H. and Z. I. KERTESZ, Bot. Gaz. 108 (1946) 279.
184 GROSSMANN, F., Naturwiss. 45 (1958) 113.
185 GYER, R. B., W. M. MILLER, O. W. BISSETT and M. K. VELDHUIS, Food Technol. 10 (1956) 10.

H

186 HAAN, I. DE, South Afric. Ind. Chemist. 35 (1957, febr.) 26.
187 HAAS-SCHULZ, E., Makromol. Chem. 7 (1951) 140.
188 HALLER, M. H., J. agric. Res. 39 (1931) 739.
189 HAMILTON, I. R. and R. A. JOHNSTON, Appl. Microbiol. 9 (1961) 121, 128.
190 HAMM, R., in: Recent advances in food science-3, Butterworths, London, (1963) 218.
191 HANSEN, E., Plant Physiol. 14 (1939) 145.
192 HARTMANN, C., Fruits 12 (1957) 45.
193 HARVEY, H. G., J. Sci. Food Agr. 1 (1950) 307.
194 HARVEY, H. G., in: Texture in foods, S. C. I. Monograph no. 7, Soc. Chem. Ind., London (1960) 29.
195 HAVAS, M., Zschr. Lebensm. unters. u. Forsch. 113 (1960) 249 (Abstr.).
196 HEIMANN, W., K. WUCHERPFENNIG and H. J. REINTJES, Zschr. Ernährungswiss. 1 (1960) 125.
197 HELBERT, J. R. and K. D. BROWN, Anal. Chem. 28 (1956) 1098.
198 HELBERT, J. R. and K. D. BROWN, Anal. Chem. 29 (1957) 1464.
199 HENGLEIN, F. A., Makromol. Chem. 1 (1943) 121.
200 HENGLEIN, F. A., Makromol. Chem. 1 (1947) 70.
201 HENGLEIN, F. A., Angew. Chem. 62 (1950) 27.
202 HENGLEIN, F. A. and B. VOLLMERT, Makromol. Chem. 2 (1948) 77.
203 HENGLEIN, F. A., H. KRÄSSIG and A. STEINMIG, Makromol. Chem. 4 (1949) 78.
204 HENGLEIN, F. A. and I. KROHN, Makromol. Chem. 4 (1949) 308.
205 HENGLEIN, F. A. and E. ANDERS, Makromol. Chem. 7 (1951) 177.
205a HERI, W., Chromatographische Fraktionierung von Pektinstoffen an Diäthylaminoäthylcellulose, Ph-D. Thesis Eidgen. Techn. Hochschule, Zürich (1962).
206 HERI, W., H. NEUKOM and H. DEUEL, Helv. Chim. Acta 44 (1961) 1939.
207 HERRMANN, J., and H. G. GROSSMANN, Pharmazeutische Zentralhalle 101 (1962) 743.
208 HES, H. N., Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, Rep. no. 1437 (1964).
209 HILLS, C. H., J. W. WHITE and G. L. BAKER, Proc. Inst. Food Techn. (1942) 47.
210 HILLS, C. H. and H. H. MOTTERN, Ind. Eng. Chem. 28 (1946) 1153.
211 HILLS, C. H. and R. SPEISER, Science 103 (1946) 166.
212 HILLS, C. H., H. H. MOTTERN, G. C. NUTTING and R. SPEISER, Food Technol. 3 (1949) 90.
213 HINTON, C. L., Fruit pectins, their chemical behaviour and jellying properties, Dept. Sci. Ind. Res., Spec. Rep. No. 48 (1939).

214 HINTON, C. L., Biochem. J., 34–2 (1940) 1211.
215 HINTON, C. L., J. Sci. Food. Agr. 1 (1950) 300.
216 HIRST, E. L. and J. K. N. JONES, J. Chem. Soc. (1938–1) 496.
217 HIRST, E. L. and J. K. N. JONES, J. Chem. Soc. (1939–1) 452.
218 HOBSON, G. E., Nature 195 (1962) 804.
219 HOBSON, G. E., Biochem. J. 86 (1963) 358.
220 HOBSON, G. E., J. Sci. Food Agric. 14 (1963) 550.
221 HOLGATE, K. and Z. I. KERTESZ, Fruit Prod. J. 28 (1949, no. 2) 37.
222 HOOGZAND, C. and J. A. S. MOERMAN, Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, Rep. No. 940 (1958).
223 HOOGZAND, C. and J. J. DOESBURG, Food Technol. 15 (1961) 160.
224 HOOVER, M. W., Food Technol. 14 (1960) 437.
225 HOTTENROTH, B., Die Pektine und Ihre Verwendung, Verl. R. Oldenbourg, München (1951).
226 HOTTENROTH, B., Zschr. Lebensm. untersuch. u. Forsch. 94 (1952) 11.
226a HSU, C. P., S. N. DESHPANDE and N. W. DESROSIER, J. Food Sci. 30 (1965) 583.
227 HULME, A. C., Adv. Food Res. 8 (1958) 297.

I

228 IFT Committee on Pectin Standardization, Food Technol. 13 (1959) 496.
229 ISHERWOOD, F. A., Food Manufact. 30 (1955) 399.

J

230 JAKOVLIV, G., Ind. agr. alim. 66 (1949) 47.
231 JAKOVLIV, G., Ind. agr. alim. 69 (1952) 317.
232 JAKOVLIV, G. and A. I. COLPE, Le Fruit Belge 19 (1951) 179.
233 JANSEN, E. F., L. R. MACDONNEL and R. JANG, Arch. Biochem. 8 (1945) 97.
234 JANSEN, E. F., L. R. MCDONNEL and W. H. WARD, Arch. Biochem. 21 (1949) 149.
235 JERMYN, M. A. and F. A. ISHERWOOD, Biochem. J. 64 (1956) 123.
236 JOHAR, D. S., G. V. KRISHNAMURTHY and B. S. BATHIA, Food Science 9 (1960) 82.
237 JOHNSON, G. and M. M. BOGGS, Food Inds 19 (1947) 1491.
238 JONES, J. K. N., Chem. & Ind. 1951, 430.
239 JONES, A. H., Rep. Canad. Comm. Fruit and Veg. Pres. 1959 (1960) 20–1.
240 JOSEPH, G. H., J. Soc. Chem. Ind. 49 (1930) 159.
241 JOSEPH, G. H., PECTIN, L. M., California Fruit Growers Exchange, Ontario, California (1947).
242 JOSEPH, G. H. and W. E. BAIER, Food Technol. 3 (1949) 18.
243 JOSEPH, G. H., A. H. KIESER and E. F. BRYANT, Food Technol. 3 (1949) 85.
244 JOSEPH, G. H. and C. R. HAVIGHORST, Food Engng (1952) 87, 134, 160, 161.
245 JOSLYN, M. A., Adv. Food Res. 11 (1962) 1.
246 JOSLYN, M. A., and A. SEDKY, Plant Physiol. 15 (1940) 675.
247 JOSLYN, M. A. and H. J. PHAFF, Wallerstein Labs Commun. 10 (1947) 39.
248 JOSLYN, M. A. and H. J. PHAFF. Wallerstein Labs Commun. 10 (1947) 133.
249 JOSLYN, M. A., S. MIST and E. LAMBERT, Food Technol. 6 (1952) 133.
250 JOSLYN, M. A. and H. DEUEL, J. Food Sci. 28 (1963) 65.
251 JOUX, J. L. Compt. rend. Acad. Agric. France 43 (1957) 506.
252 JOUX, J. L., Compt. rend. Acad. Agric. France 43 (1957) 807.

K

253 KACZMARZYK, L. M., O. FENNEMA and W. D. POWRIE, Food Technol. 17 (1963) 943.
254 KAJI, A., Bull. Agric. Chem. Soc. Japan, 23–2 (1959) 131.
255 KEFFORD, J. F., Adv. Food Res. 9 (1959) 285.
256 KERR, T., Growth and structure of the primary wall, in: 'Plant growth substances', edited by F. Skoog, Univ. of Wisconsin Press (1951).
257 KERTESZ, Z. I., J. biol. Chem. 121 (1937) 589.
258 KERTESZ, Z. I., Plant Physiol. 18 (1943) 308.

259 KERTESZ, Z. I., The pectic substances, Intersci. Publ., New York–London (1951).
260 KERTESZ, Z. I., in: Food enzymes, edited by H. W. Schultz, Avi Publishing Cy, Westport, Connecticut (1960) 49.
261 KERTESZ, Z. I., and E. L. GREEN, N.Y. State Agric. Exp. Sta., Techn. Bull. No. 179 (1931).
262 KERTESZ, Z. I., G. L. BAKER, G. H. JOSEPH, H. H. MOTTERN and A. G. OLSEN, Chem. Eng. News 22 (1944) 105.
263 KERTESZ, Z. I. and R. J. MCCOLLOCH, N. Y. St. Agric. Exp. Sta., Bull. No. 745 (1950).
264 KERTESZ, Z. I., B. H. MORGAN, L. W. TUTTLE and M. LAVIN, Radiation Res. 5 (1956) 372.
265 KERTESZ, Z. I., M. EUCARE and G. FOX, Food Research 24 (1959) 14.
266 KERTESZ, Z. I., R. E. GLEGG, F. P. BOYLE, G. F. PARSONS and L. M. MASSEY, J. Food Sci. 29 (1964) 40.
267 KIEFER, F., Food Technol. 15 (1961) 302.
268 KIESER, M. E., A. POLLARD and A. M. STONE, Ann. Rep. Long Ashton Res. Sta. for 1949, 153.
269 KIESER, M. E., A. POLLARD and D. J. SISSONS, Ann. Rep. Long Ashton Res. Sta. (1957) 134.
270 KOCH, J., Fruchtsaft Ind. 1 (1956) 66.
271 KOCH, J. and G. BRETTHAUER, Zschr. Lebensm. unters. u. Forsch. 95 (1952) 225.
272 KREBS, J., Flüss. Obst 22 (1955, no. 6) 6.
273 KREBS, J. Flüss. Obst 24 (1957, no. 6) 13.
274 KREBS, J., Conserva, 11 (1962/63) 258.

L

275 LAMPI, R. A., W. B. ESSELEN, C. L. THOMSON and E. E. ANDERSON, Food Research 23 (1958) 351.
276 LAMPITT, L. H. and R. W. MONEY, J. Soc. Chem. Ind. 56 (1937) 290 T.
277 LANGE, D., W. BOCK and K. TÄUFEL, Ernährungsforsch. 5 (1960) 18.
278 LANGE, D., W. BOCK and K. TÄUFEL, Ernährungsforsch. 6 (1961) 65.
279 LARSEN, H. Food Technol. in Austr. 11 (1959) 111.
280 LAWRENCE, J. M. and K. GROVES, J. Agr. Food Chem. 2 (1954) 882.
281 LEFÈVRE, K. N. and B. TOLLENS, Ber. deutsch. chem. Ges. 40 (1907) 4513.
282 LEWIS, J. C., C. F. PIERSON and M. J. POWERS, Appl. Microbiol. 11 (1963) 93.
283 LINEWEAVER, H., J. Am. Chem. Soc. 67–II (1945) 1292.
284 LINEWEAVER, H., and G. A. BALLOU, Arch. Biochem. 6 (1945) 373.
285 LINEWEAVER, H. and E. F. JANSEN, Adv. Enzymol 11 (1961) 267.
286 LOCONTI, J. D. and Z. I. KERTESZ, Food Research 6 (1941) 449.
287 LOTZKAR, H., T. H. SCHULTZ, H. S. OWENS and W. D. MACLAY, J. phys. Chem. 50 (1946) 200.
288 LOWINGS, P. H., Appl. Microbiol. 4 (1956) 84.
289 LÜDTKE, M. and H. FELSER, Ann. 549 (1941) 1.
290 LÜERS, H. and K. LOCHMÜLLER, Kolloïd-Zschr. 42 (1957) 154.
291 LUH, B. S. and H. J. PHAFF, Arch. Biochem. Biophys. 51 (1954) 102.
292 LUH, B. S., S. J. LEONARD and H. J. PHAFF, Food Research 21 (1956) 448.
293 LUH, B. S., F. VILLARREAL, S. J. LEONARD and M. YAMAGUCHI, Food Technol. 14 (1960) 635.

M

294 MAASS, H., Die Pektine, Serger and Hempel, Braunschweig (1951).
295 MAGNESS, J. R. and G. F. TAYLOR, U.S. Dept. Agr., Dept. Circ. 350 (1925).
296 MANGIN, L., Compt. rend. 111 (1890) 120.
297 MANGIN, L., Compt. rend. 116 (1893) 653.
298 MANHEIM, H. C. and S. ZIV, Abstr. 1st Int. Congr. Food Sci. Technol., London (1962) 65.
299 MARTIN, D. Aust. J. Agric. Res. 5 (1954) 9.
300 MARTIN, C. M., Food Technol. in Austr. 13 (1961) 420.
301 MATELES, R. I., F. H. DEINDORFER and A. E. HUMPHREY, Biotechn. and Bioeng. 6 (1964) 5.
302 MATTSON, S., Acta agric. Suec. 2 (1946) 185.
303 MATTSON, S., E. ÅKERBERG, E. ERIKSON, E. D. KOUTLER-ANDERSON and K. VAHTRAS, Act. agric. Scand. 1 (1951) 40.

304 MATUS, J., Ber. Schweiz Bot. Ges. 58 (1948) 319.
305 MATZ, S. A., Food texture, Avi Publ. Cy. Inc., Westport, Connecticut (1962).
306 MCARDLE, F. J. and J. V. NEHEMIAS, Food Technol. 10 (1956) 599.
307 MCCLENDON, J. H. and G. F. SOMMERS, Plant Phys. 31 (1956) suppl. xlii.
308 MCCLENDON, J. H., C. W. WOODMANSEE and G. F. SOMERS, Plant Physiol. 34 (1959) 389.
309 MCCLENDON, J. H. and J. L. HESS, J. Food Sci. 28 (1963) 289.
310 MCCOLLOCH, R. J. and Z. I. KERTESZ, J. biol. Chem. 160 (1945) 149.
311 MCCOLLOCH, R. J. and Z. I. KERTESZ, Arch. Biochem. 13 (1947) 217.
312 MCCOLLOCH, R. J. and Z. I. KERTESZ, Arch. Biochem. 17 (1948) 197.
313 MCCOLLOCH, R. J. and Z. I. KERTESZ, Nature 17 (1948) 197.
314 MCCOLLOCH, R. J. and Z. I. KERTESZ, Food Technol. 3 (1949) 94.
315 MCCOLLOCH, R. J., B. W. NIELSEN and E. A. BEAVENS, Food Technol. 4 (1950) 339.
316 MCCOLLOCH, R. J., G. J. KELLER and E. A. BEAVENS, Food Technol. 6 (1952) 197.
317 MCCOLLOCH, R. J., R. G. RICE, B. GENTILI and E. A. BEAVENS, Food Technol. 10 (1956) 633.
318 MCCOMB, E. A. and R. M. MCCREADY, Anal. Chem. 24 (1952) 1630.
319 MCCREADY, R. M., A. D. SHEPHERD and W. D. MACLAY, Fruits Prod. J. 26 (1942) 36.
320 MCCREADY, R. M., H. S. OWENS and W. D. MACLAY, Food Inds 16 (1944) 794, 864, 906.
321 MCREADY, R. M., H. A. SWENSON and W. D. MACLAY, Ind. Eng. Chem., Anal. Ed. 18 (1946) 290.
322 MCCREADY, R. M., H. OWENS, A. D. SHEPHERD and W. D. MACLAY, Ind. Eng. Chem. 38 (1946) 1254.
323 MCCREADY, R. M., A. D. SHEPHERD, H. A. SWENSON, R. A. ERLANDSEN and W. D. MACLAY, Anal. Chem. 23 (1951) 975.
324 MCCREADY, R. M. and E. A. MCCOMB, Anal. Chem. 24 (1952) 1986.
325 MCCREADY, R. M. and C. G. SEEGMILLER, Arch. Biochem. Biophys. 50 (1954) 440.
326 MCCREADY, R. M. and E. A. MCCOMB, Food Research 19 (1954) 530.
327 MCCREADY, R. M. and R. M. REEVE, J. Agr. Food Chem. 3 (1955) 260.
328 MCCREADY, R. M., E. A. MCCOMB and E. F. JANSEN, Food Research 20 (1955) 186.
329 MCCREADY, R. M. and M. GEE, J. Agr. Food Chem. 8 (1960) 510.
330 MCDONNEL, L. R., E. F. JANSEN and H. LINEWEAVER, Arch. Biochem. 6 (1945) 389.
331 MCDONNEL, L. R., R. JANG, E. F. JANSEN and H. LINEWEAVER, Arch. Biochem. 28 (1950) 260.
332 MCGREGOR, D. R., Rep. Canad. Comm. Fruit and Veg. Pres. 1959 (1960) 17.
333 MEHLITZ, A., Vorratspflege u. Lebensm. forsch. 4 (1941) 572.
334 MEHLITZ, A., Ind. Obst. u. Gemüseverwert. 35 (1950) 127, 142, 162, 182, 200, 219, 237.
335 MERRIL, R. C. and M. WEEKS, J. Am. Chem. Soc. 67 (1945) 2244.
336 MERRIL, R. C. and M. WEEKS, J. phys. Chem. 50 (1946) 75.
337 MESCHTER, E. E. and L. J. LATAILLADE, Food Technol. 3 (1949) 28.
338 MOGHRABI, H. M., Diss. Abstr. 19 (1958) 928.
339 MOORE, E. L., A. H. ROUSE and C. D. ATKINS, Food Technol. 16 (1962, no. 12) 91.
340 MORRIS, T. N., Principles of fruit preservation, Chapman and Hall, London, (1946).
341 MOTTERN, H. H., Food Technol. 3 (1949) 34.
342 MOTTERN, H. H. and E. KARR, Fruit Prod. J. (1946) 292.
343 MOTTERN, H. H. and C. H. HILLS, Ind. Eng. Chem. 38 (1946) 1153.
344 MÜHLETAHLER, K., Biochim. Biophys. Acta 5 (1950) 1.
345 MUNDT, J. O. and I. E. MCCARTY, Food Technol. 14 (1960) 309.

N

346 NEUKOM, H., Studien über das Geliervermögen von Pektinstoffen und anderen hochmolekularen Polyoxyverbindungen, Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1949).
347 NEUKOM, H., Chimia 14 (1960) 165.
348 NEUKOM, H. and H. DEUEL, Chem. & Ind. (1958) 683.
349 NEUKOM, H. and H. DEUEL, Beiheft Zschr. Schweiz Forstv. 30 (1960) 223.
350 NEUKOM, H., H. DEUEL, W. J. HERI and W. KÜNDIG, Helv. Chim. Acta 43 (1960) 64.
351 NEWBOLD, R. P. and M. A. JOSLYN, J. Assoc. Offic. Agr. Chem. 35 (1952) 872.
352 NORTHCOTE, D. H., Biol. Rev. 33 (1958) 53.

O

353 OLLIVER, M., J. Sci. Food Agric. 1 (1950) 329.
354 OLLIVER, M., Food Technol. 4 (1950) 370.
355 OLLIVER, M., P. WADE and K. P. DENT, Analyst 82 (1957) 127.
356 OLLIVER, M., P. WADE and K. P. DENT, J. Sci. Food Agric. 8 (1957) 188.
357 OLSEN, A. G., J. phys. Chem. 38 (1934) 919.
358 OLSEN, A. G. and R. STUEWER, U.S. Patent no. 2, 132, 577 (1938) October 11.
359 OLSEN, A. G., R. F. STUEWER, E. R. FEHLBERG and N. M. BEACH, Ind. Eng. Chem. 31 (1939 1015.
360 OLTHOF, E., Conserva 9 (1960/61) 306.
361 OTT, A. C. and C. D. BALL, Arch. Biochem. 3 (1943) 189.
362 OWENS, H. S., O. PORTER and W. D. MACLAY, Food Inds 19 (1947) 606.
363 OWENS, H. S., R. M. MCCREADY and W. D. MACLAY, Food Technol. 3 (1949) 77.
364 OWENS, H. S., R. M. MCCREADY, A. D. SHEPHERD, T. H. SCHULTZ, E. L. PIPPEN, H. A. SWENSON, J. C. MIERS, R. F. ERLANDSEN and W. D. MACLAY, Methods used at Western Regional Research Laboratory for extraction and analysis of pectic materials, Albany, California, U.S. Dept. of Agr., A.I.C.-340 (mimeographed) (1952).
365 OZAWA, J. and K. OKAMOTO, Ber. Ohara Inst. 10 (1955) 215; Food Sci. Abstr. 28 (1956) No. 316.

P

366 PALLMANN, H., F. WEBER and H. DEUEL, Schweiz. landwirtschaftl. Monatsh. 22 (1944) 306.
367 PALLMANN, H. and H. DEUEL, Experientia 1 (1945) 89.
368 PALLMANN, H. and H. DEUEL, Chimia 1 (1947) 27.
369 PALMER, K. J. and M. B. HARTZOG, J. Am. Chem. Soc. 67 (1945–2) 2122.
370 PANDHI, P. N., British Food Manuf. Ind. Res. Ass., Sci. and Techn. Surveys no. 22 (1953).
371 Pectin Sub-committee of the Jam Panel, Brit. Food Manuf. Ind. Res. Ass., Analyst 76 (1951) 536.
372 PERSONIUS, C. J. and P. F. SHARP, Food Research 3 (1938) 513.
373 PERSONIUS, C. J. and P. F. Sharp, Food Research 4 (1939) 469.
374 PETERS, G. L., H. D. BROWN, W. A. GOULD and R. B. DAVIS, Food Technol. 8 (1954) 220.
375 PEYNAUD, E., Ind. agr. alim. 68 (1951) 609.
376 PILNIK, W., in: Fruit juice concentrates. Intern. Fed. Fruit Juice Producers, Juris Verlag, Zürich (1958) 203.
377 PILNIK, W., Fruchtsaft Ind. 5 (1964) 277.
377a PILNIK, W., personal communication (1964).
378 PILNIK, W. and G. ROTHSCHILD, Bull. Res. Counc. Israel 4 (1954) 63.
379 PILNIK, W. and G. ROTHSCHILD, Fruchtsaft Ind. 5 (1960) 131.
380 PIPPEN, E. L., R. M. MCCREADY and H. S. OWENS, J. Am. Chem. Soc. 72 (1950) 813.
381 PIPPEN, E. L., R. M. MCCREADY and H. S. OWENS, Anal. Chem. 22 (1950) 1457.
382 POLLARD, A. and M. E. KIESER, J. Sci. Food Agr. 2 (1951) 30.
383 POLLARD, A. and M. E. KIESER, J. Sci. Food Agr. 10 (1959) 253.
384 PORRETTA, A. and V. CASOLI, Industria Conserva 36 (1961) 104.
385 PORTER, W. L., J. H. SCHWARTZ, T. A. BELL and J. L. ETCHELLS, J. Food Sci. 26 (1961) 600.
386 PORTER, W. L. and J. H. SCHWARTZ, J. Food Sci. 27 (1962) 416.
387 POSTLMAYR, H. L., B. S. LUH and S. J. LEONARD, Food Technol. 10 (1956) 618.
388 PRATT, D. E. and J. J. POWERS, Food Research 18 (1953) 152.
389 PROEBSTING, E. L., G. H. CARTER, D. W. INGASLBE and A. M. NEUBERT, Proc. Am. Soc. Hort. Sci. 69 (1957) 131.

R

390 RAHMAN, M. B. and M. A. JOSLYN, Food Research 18 (1953) 301.
391 RANGANA, S., M. V. SASTRY and G. S. SIDDAPPA, Confructa 1 (1961) 5.
392 REEVE, R. M., Food Research 12 (1947) 10.
393 REEVE, R. M., Food Research 18 (1953) 604.
394 REEVE, R. M., Am. J. Bot. 46 (1959) 241.

395 REEVE, R. M., Stain Technol. 34 (1959) 209.
396 REEVE, R. M. and L. R. LEINBACH, Food Research 18 (1953) 592.
397 REEVE, R. M. and C. H. H. NEUFELD, Food Research 24 (1959) 552.
398 REID, W. W., Nature 166 (1950) 76.
399 REID, W. W., J. Sci. Food Agr. 1 (1950) 324.
400 REID, W. W., in: Recent advances in fruit juice production, edited by V. L. S. Charley, Techn. Commun. Bur. Hort., E. Malling No. 21 (1950) 145.
401 REID, W. W., in: Production and application of enzyme preparations in food manufacture, S.C.I. Monograph No. 11, Soc. Chem. Ind., London (1961) 35.
402 RENTSCHLER, H., Schweiz. Z. Obst. Weinb. 60 (1951) 282.
403 RHODES, W. E., and H. F. DAVIES, Chem. & Ind. 21 (1945) 162.
404 ROBOZ, E., R. W. BARRATT and E. L. TATUM, J. biol. Chem. 195 (1952) 459.
405 ROELOFSEN, P. A., Biochim. Biophys. Acta 10 (1953) 410.
406 ROELOFSEN, P. A., Acta Botan. Neerl. 3 (1954) 154.
407 ROELOFSEN, P. A., The Plant Cell Wall; Handb. Pflanzenanat. III/4, Gebr. Borntraeger, Berlin-Nikolassee (1959).
408 ROELOFSEN, P. A. and D. R. KREGER, J. exptl. Bot. 2 (1951) 332.
409 ROELOFSEN, P. A. and D. R. KREGER, J. exptl. Bot. 5 (1954) 24.
410 ROUSE, A. H. and C. D. ATKINS, Food Technol. 6 (1952) 291.
411 ROUSE, A. H. and C. D. ATKINS, Proc. Florida State Hort. Soc. 66 (1953) 268.
412 ROUSE, A. H., C. D. ATKINS and R. L. HUGGART, Food Technol. 8 (1954) 431.
413 ROUSE, A. H., C. D. ATKINS and E. L. MOORE, J. Food Sci. 27 (1962) 419.
414 ROUSE, A. H., C. D. ATKINS and E. L. MOORE, J. Food Sci. 29 (1964) 34.

S

415 SABOUROFF, N. V. and M. I. KALEBIN, Fruit Prod. J. 14 (1935), 275, 280.
416 SÄVERBORN, S., A contribution to the knowledge of the acid polyuronides, Ph-D. Thesis Univ. Uppsala (1945).
417 SAYRE, C. B. and B. R. NEBEL, Proc. Am. Soc. Hort. Sci. 27 (1930) 221.
418 SCHILT, R., Pectinen en pectinesplitsende enzymen, Ph-D. Thesis Univ. Utrecht (1961).
419 SCHLUBACH, H. H. and H. P. HOFFMANN-WALBECK, Makromol. Chem. 4 (1949) 5.
420 SCHNEIDER, G. G. and U. FRITCHI, Ber. deut. chem. Ges. 69 (1936) 2537.
421 SCHNEIDER, G. G. and H. BOCK, Ber. deut. chem. Ges. 54 (1937) 1860.
422 SCHNEIDER, G. G. and H. BOCK, Z. Angew. Chem. 51 (1938) 94.
423 SCHUBERT, E., Schweiz. Brau. Rundsch. 62 (1951) 39.
424 SCHUBERT, E., Bioch. Zschr. 323 (1952) 78.
425 SCHUBERT, E., Schweiz. Brau. Rundsch. 63 (1952) 95.
426 SCHULTZ, T. H., H. LOTZKAR, H. S. OWENS, and W. D. MACLAY, J. phys. Chem. 49 (1945) 554.
427 SCHULTZ, T. H., H. S. OWENS and W. D. MACLAY, J. Colloid Sci. 3 (1948) 53.
428 SEEGMILLER, E. G. and E. F. JANSEN, J. biol. Chem. 195 (1952) 327.
429 SERGER, H., Obst. u. Gemüse Verw. ind. 29 (1942) 186.
429a SHEWFELT, A. L., 24th Ann. Meeting Inst. Food Technol. Washington (1964) 46, no. 35.
430 SHIMAZU, F. and C. STERLING, J. Food Sci. 26 (1961) 291.
431 SHIOIRI, H. and I. HAGINUMA, Chem. Abstr. 53 (1959) 11707.
432 SIMPSON, J. L. and E. G. HALLIDAY, Food Research 6 (1941) 189.
433 SINCLAIR, W. B. and P. R. CRANDALL, Plant Physiol. 24 (1949) 681.
434 SINCLAIR, W. B. and P. R. CRANDALL, Bot. Gaz. 111 (1949) 153.
435 SINCLAIR, W. B. and P. R. CRANDALL, Bot. Gaz. 115 (1953) 162.
436 SINCLAIR, W. B. and V. A. JOLIFFE, J. Food Sci. 26 (1961) 125.
436a SISTRUNK, W. A., J. Food Sci. 30 (1965) 240.
437 SISTRUNK, W. A. and R. F. CAIN, Food Technol. 14 (1960) 357.
438 SISTRUNK, W. A., R. F. CAIN, E. K. VAUGHAN and H. B. LAGERSTEDT, Food Technol. 14 (1960) 640.
439 SLAVIČKOVÁ, A., Sci. Papers Inst. Chem. Technol., Prague, Food Technol. 5 (1961) 73.
440 SLOEP, A. C., Onderzoekingen over pectinestoffen en hare enzymatische ontleding, Ph-D. Thesis, Delft (1928).

441 SMOCK, R. M. and A. M. NEUBERT, Apples and apple products, Intersci. Publ. New York–London (1950).
442 SMYTHE, C. V., B. B. DRAKE and J. A. MILLER, U.S. Patent 2599531, 10th June 1952; Chem. Abstr. 46 (1952) 9664 i.
443 SNYDER, E. B., Res. Bull. Neb. Agric. Exp. Sta. No. 85 (1936).
444 SOLMS, J., Schweiz. Zschr. f. Obst. und Weinbau 63 (1954) 319.
445 SOLMS, J. and H. DEUEL, Helv. Chim. Acta 34 (1951) 2242.
446 SOLMS, J., A. DENZLER and H. DEUEL, Helv. Chim. Acta 37 (1954) 2155.
447 SOLMS, J. and H. DEUEL, J. Polym. Sci. 12 (1954) 559.
448 SOLMS, J. and H. DEUEL, Helv. Chim. Acta, 38 (1955) 321.
449 SOMOGYI, L. P. and R. J. ROMANI, J. Food Sci 29 (1964) 366.
450 SPEISER, R., C. R. EDDY and C. H. HILLS, J. phys, Chem. 49 (1945) 563.
451 SPEISER, R., M. J. COPLEY and G. C. NUTTING, J. Phys. Colloid Chem. 51 (1946) 117.
452 SPENCER, G. J. phys. Chem. 34 (1930) 429.
453 STADEN, O. L. and J. J. DOESBURG, Ann. Rep. Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, (1960) 53.
454 STADEN, O. L. and J. J. DOESBURG, Ann. Rep. Inst. Res. Stor. & Proc. Hort. Prod., Wageningen, Netherlands, (1961) 60.
455 STEELE, W. F. and H. Y. YANG, Food Technol. 14 (1960) 121.
456 STERLING, C., Food Research 20 (1955) 474.
457 STERLING, C., Food Technol. 13 (1959) 629.
458 STERLING, C., J. Food Sci. 26 (1961) 95.
459 STERLING, C. and A. J. KALB, Bot. Gaz. 121 (1959) 111.
460 STERLING, C. and C. O. CHICHESTER, Food Research 25 (1960) 157.
461 STERLING, C. and F. A. BETTELHEIM, Food Research 25 (1960) 130.
462 STERLING, C. and F. SHIMAZU, J. Food Sci. 26 (1961) 479.
463 STEVENS, J. W., D. E. PRITCHETT and W. E. BAIER, Food Technol. 4 (1950) 469.
464 STOIKOFF, S., Mitt. Geb. Lebensm. Hyg. 39 (1948) 292.
465 STUEWER, R., N. M. BEACH and A. G. OLSEN, Ind. Eng. Chem., Anal. Ed. 6 (1934) 143.
466 STUTZ, E., Dehydratisierung und Dekarboxylierung von Uronsäuren, Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1958).
467 STUTZ, E. and H. DEUEL, Helv. Chim. Acta 41 (1958) 1722.
468 SUCHARIPA, R., J. Assoc. Offic. Agr. Chem. 7 (1923) 57.
469 SULČ, D. and M. RISTIČ, Zschr. Lebensm. unters. u. Forsch. 98 (1954) 430.
470 SWAJKAJZER, A., Brit. Food Manuf. Ind. Res. Ass., Ann. Rep. (1962) 29.
471 SWENSON, H. A., T. C. SCHULTZ and H. S. OWENS, J. Agric. Food Chem. 1 (1953) 616.

T

472 TARR, L. W., Delaware Agr. Expt. Sta. Bull. 142 (1926).
473 TÄUFEL, K. and F. BERSCHNEIDER, Nahrung 3 (1959) 228.
474 TÄUFEL, K. and W. BOCK, Ernährungsforsch. 6 (1961) 553.
475 TETLEY, U., J. Pom. Hort. Sci. 8 (1930) 153.
476 TIBENSKY, V., J. ROSÍK, and V. ZITKO, Nahrung 7 (1963) 321.
477 TOLDBY, V. and R. C. WILEY, Proc. Am. Soc. Hort. Sci. 81 (1962) 78.
478 TRACEY, M. V., Biochem. J. 43 (1948) 185.
479 TRESSLER, D. K. and C. F. EVERS, The freezing preservation of foods, 3rd Edition, Avi Publ. Cy., Westport, Connecticut (1957).
480 TUTIN, F., Biochem. J. 17 (1923) 511.

U

481 ULRICH, R., VIIIth Intern. Congr. Refrigeration, London (1951).
482 ULRICH, R., La vie des fruits, Masson et Cie, Paris (1952).
483 ULRICH, R., Ann. Rev. Plant Physiol. 9 (1958) 385.
484 ULRICH, R., J. RENAC and J. MIMAULT, C. r. Acad. Agric. Fr. 38(1952) 242.
485 ULRICH, R. and J. MIMAULT, Fruits 7 (1952) 273.

486 ULRICH, R., J. RENAC and J. MIMAULT, Rev. Gén. Froid 29 (1952) 1213.
487 ULRICH, R., A. PAULIN and J. MIMAULT, Rev. Gén. Froid 31 (1954) 675.
488 ULRICH, R. and J. MIMAULT, Fruits 11 (1956) 467.

V

489 VAJDA, A., Gewinnung und Eigenschaften von Oxypropylestern der Pektinsäure, Ph-D. Thesis Eidgen. Tech. Hochsch. Zürich (1957).
490 VAN BUREN, J. P., J. C. MOYER, D. E. WILSON, W. B. ROBINSON and D. B. HAND, Food Technol. 14 (1960) 233
491 VAN BUREN, J. P., J. C. MOYER, W. B. ROBINSON and D. B. HAND, Hoppe Seyler's Zschr. physiol. Chem. 321 (1960) 107.
492 VAN BUREN, J. P., J. C. MOYER and W. B. ROBINSON, J. Food Sci. 27 (1962) 291.
493 VAN BUREN, J. P. and N. H. PECK, Proc. Am. Soc. Hort. Sci. 82 (1963) 316.
494 VAUQUELIN, M., Ann. chim. et phys. 5 (1790) 92.
495 VITINKA, J., M. ROSA and F. STROS, Fruchtsaft Ind. 6 (1961) 99.
496 VOLLMERT, B., Angew. Chem. 59 (1947) 177.
497 VOLLMERT, B., Zschr. Lebensm. untersuch. u. Forsch. 89 (1949) 347.
498 VOLLMERT, B., Makromol. Chem. 5 (1950) 101.
499 VOLLMERT, B., Makromol. Chem. 5 (1950) 128.

W

500 WADE, P., J. Sci. Food Agr. 15 (1964) 51.
501 WAGER, H. G., J. Sci. Food Agr. 14 (1963) 583.
502 WAGGONER, P. E. and A. E. DIMOND, Phytopathol. 45 (1955) 79.
503 WARD, W. H., H. A. SWENSON and H. S. OWENS, J. Phys. Coll. Chem. 51 (1947) 1137.
504 WEBER, F. and H. DEUEL, Mitt. Geb. Lebensm. Hyg. 36 (1945) 368.
505 WEIER, T. and C. R. STOCKING, Adv. Food Res. 2 (1949) 297.
506 WENZEL, F. W., E. L. MOORE, A. H. ROUSE and C. D. ATKINS, Food Technol. 5 (1951) 454.
507 WEURMAN, C., Pectine-omzettingen en pectine-fermenten in Doyenné Boussoch peren, Ph-D. Thesis. Univ. Amsterdam (1952).
508 WEURMAN, C., Acta Botan. Neerl. 2 (1953) 107.
509 WEURMAN, C., Acta Botan. Neerl. 3 (1954) 100.
510 WEURMAN, C., Acta Botan. Neerlandica, 3 (1954) 108.
511 WHISTLER, R. L., A. R. MARTIN and M. HARRIS, J. Res. Nat. Bur. Stand. 24 (1940) 13.
512 WHITE, L. S. and F. W. FABIAN, Appl. Microbiol. 1 (1953) 243.
513 WHITTENBERGER, R. T. and C. H. HILLS, Food Technol. 7 (1953) 29.
514 WHITTENBERGER, R. T. and G. C. NUTTING, Food Technol. 11 (1957) 19.
515 WHITTENBERGER, R. T. and G. C. NUTTING, Food Technol. 12 (1958) 420.
516 WICHMANN, H. J., J. Assoc. Offic. Agr. Chem. 6 (1922) 34.
517 WIDDOWSON, E. M., Ann. Bot. 36 (1932) 597.
518 WIERINGA, K. T., Conserva 1 (1952/53) 54.
519 WILEY, R. C. and G. HENBRIDGE, Proc. Am. Soc. Hort. Sci. 77 (1961) 60.
520 WOOD, R. K. S., A. H. GOLD and T. E. RAWLINS, Am. J. Bot. 39 (1952) 132.
521 WOODMANSEE, C. W. and G. L. BAKER, Food Technol. 3 (1949) 82.
522 WOODMANSEE, C. W., J. H. MCCLENDON and G. F. SOMERS, Food Research 24 (1959) 503.

Y

523 YANG, H. Y., W. F. STEELE and D. J. GRAHAM, Food Technol. 14 (1960) 644.
524 YÚFERA, E. P., J. K. MOSSE and J. ROYO IRANZO, Abstr. 1st Int. Congr. Food Sci. Technol. London (1962) 52.
525 YÚFERA, E. P., J. K. MOSSE and J. ROYO IRANZO, Rev. Agroquim. Technol. Alim. 3 (1963) 9.

Z

526 ZIEGELMAYER, W., Molkerei Zeit. 48 (1934) 495.
527 ZITKO, V. and J. ROSIK, Nahrung 6 (1962) 560.

528 ZITKO, V., J. ROSIK and J. VASATKO, Fruchtsaft Ind. 3 (1963) 142. Abstr.
528a ZWEEDE, A. K., Conserva 3 (1954/55) 18.
529 ZWEIFEL, G. Ueber die Dekarboxylierung von Uronsäuren, Ph-D. Thesis Eidgen. Techn. Hochsch. Zürich (1956).

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