Studies on cloud stability of apricot nectar

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Abstract

Siliha, H.A.I. (1985) Studies on cloud stability of apricot nectar. Doctoral thesis, Wageningen. (x) + 104 p., 41 figs, 27 tables, 163 refs, Dutch and English summaries.

Cloud loss behaviour in pasteurized apricot nectar was found to be different from that of other fruit juices. The cloud particles settled slowly on standing and a gel formed. On standing for a longer period the gel contracts and a clear supernatant layer which can be considered partly as syneresis water is formed. Addition of commercial pectolytic enzyme preparations containing mainly PG activity prevented gel formation and only improved the level of stability. Wide spectrum preparations containing high activities of PE, PG, PL, and also hemicellulases, cellulases, and proteases stabilized the cloud particles completely. Cloud could also be stabilized by the addition of purified polysaccharide-degrading enzymes such as PL, PG + mould PE, and PG + exo-arabinase. Examination of the changes in the chemical composition of serum and cloud particles showed that the cloud stabilizing enzymes almost completely solubilized and degraded the arabinan-rich pectic material. Microscopic examination revealed that in cloud stable nectar the cell walls of the cloud particles were ruptured. Determination of cloud particle density and rheological measurements showed that cloud stabilizing enzymes decreased cloud particle density and that they increased the yield stress of the nectar more than did non-stabilizing enzymes. Literature on pectic substances, pectolytic enzymes, composition of cloud particles of fruit juices and nectars and on cloud stability of citrus juices, non-citrus juices and nectars have been reviewed.

Suppositions

1. There is no justification for the statement of Ishii & Yokotsuka (1972) and Reymond & Bush (1972) that for fruit processing with enzymes, pectin lyases rather than the combination of methyl esterases with polygalacturonases should be used because of the release of methanol by esterases.

Ishii, S. & T. Yokotsuka, 1972. J.agric. Food Chem. 20: 787-791
Reymond, D. & D.A. Bush, 1972. Ann. Technol. Agric. 21: 545-553.

2. The generally used German expression "Maische Fermentierung" for the enzymatic treatment of fruit and vegetable pulp is misleading.

This thesis, section 2.5.

3. The finding of Strübi (1976) that both Pectinex, which is a wide spectrum pectolytic enzyme preparation, and Irgazyme M-10, which contains mainly PG activity, stabilize the cloud of apple nectar makes his theory on cloud stability uncertain.

Strübi, P., 1976. Ph.D. Thesis, Swiss Federal Institute of Technology, Zürich.

4. From the nutritional view cloudy fruit juices and nectars have more value than clear juices.

5. The ability of bacteria to digest fully differentiated plant cell walls is not limited by the lignin content as stated by Akin *et al.* (1984), but by the presence of a warty layer.

Akin, D.E., L.L. Rigsby & R.H. Brown, 1984. Crop Science, 24: 156-162.

6. The role of hemicellulases in fruit and vegetable processing needs more attention.

This thesis, section 4.6.

7. Association of pectin chains by interaction with calcium ions in sugar beet pulp as a consequence of the liming process cannot be explained only by de-acetylation as stated by Camirand *et al.* (1981).

Camirand, W.M., J.M. Randall, E.M. Zaragosa & H. Neuman, 1981. Journal of the A.S.S.B.T. 21: 159-174.

8. It is doubtful that extensin or similar glycoproteins have an important role in the structure of cell walls of fruits and vegetables as suggested by Lamport & Epstein (1983) for the walls of tomato cells in suspension-culture.

Lamport, D.T.A. & L. Epstein, 1983. Current Topics in Plant Biochemistry and Physiology 2: 73-83.

9. For the better understanding of the fine structure of pectic substances, intensive research must be devoted to find rhamnogalacturonan depolymerases which specifically split the glycosidic bonds adjacent to rhamnose residues.

10. The differences in the stabilization and clarification mechanisms of natural fruit juices and nectars indicate that it may not be possible to postulate a general physical theory for cloud stabilization and clarification.

H.A.I. Siliha
Studies on cloud stability of apricot nectar
Wageningen, 23 april 1985

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I would like to thank Professor Dr M.M. Abd El-Baki, Dean of the Faculty of Agriculture and Professor Dr A. Askar, Chairman of the Department of Food Science and Technology, University of Suez Canal, Ismailia, Egypt, for making it possible for me to come to the Netherlands to undertake this research work. I would like also to thank Mr F.J. Meijsing and Mr C.G. Brom of the Netherlands Ministry of Education for assisting me with the arrangements for my scholarship. I would like to thank the LEB-Fonds for financial support for the printing of the manuscript.

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To
Egypt,
my parents,
my wife Hayam,
and my son Ahmed

Curriculum vitae

Hassan Ali Ibrahim Siliha was born in Alexandria, Egypt, on 5th November 1951. In 1970 he enrolled in the Faculty of Agriculture of the University of Alexandria and in 1974 graduated with honours in food science and technology. In the same year he was appointed to the position of demonstrator in the Department of Food Science and Technology in the Faculty of Agriculture, the University of Zagazig. While working in the department, he undertook studies for the degree of Master of Science which he obtained in 1977. He was then promoted to the position of assistant lecturer in the same department. In October 1980, he was awarded a scholarship by the Netherlands Ministry of Education to undertake the research presented in this thesis in the Department of Food Science, Agricultural University, Wageningen.

Samenvatting

Het doel van deze studie was de invloed van pektolytische enzymen op de troebelingsstabiliteit van abrikozenektar te onderzoeken en het mechanisme van de stroebelingsstabiliteit door middel van moderne analytische technieken op te helderen. Deze doelstellingen zijn nader uitgewerkt in hoofdstuk 1.

In hoofdstuk 2 volgt een uitvoerige bespreking van de literatuur betreffende troebelingsstabiliteit van vruchtesappen en nectars. Eerst wordt ingegaan op de chemie en enzymologie van pektinestoffen in fruit, daarna worden de diverse verschijningsvormen van troebele deeltjes in diverse vruchtesappen en nectars besproken en wordt ingegaan op hun chemische samenstelling. Vervolgens wordt de troebelingsstabiliteit van vruchtesappen en nectars behandeld in relatie tot de activiteit van pektolytische enzymen die in staat zijn troebelingen te destabiliseren (appelsap) of juist te stabiliseren (nectar). Tenslotte wordt het effekt van homogeniseren op de stabiliteit van troebele vruchte- en groentepulpen beschreven.

De in dit onderzoek gebruikte materialen en methodieken zijn beschreven in hoofdstuk 3. Speciale aandacht werd besteed aan de bepaling van het anhydrogalacturonzuurgehalte (AGA) en de veresteringsgraad (DE) van oplosbaar en onoplosbaar pektine. Daarvoor werden twee methoden gebruikt; de koperionen-uitwisselingsmethode en de colorimetrische m-hydroxydifenyl (mhdp) methode in combinatie met gaschromatografische methanolbepaling. De kopermethode gaf betrouwbare en reproduceerbare resultaten voor het AGA-gehalte in de alcohol onoplosbare bestanddelen (AIS) van fruitmengsels. Voor de wateroplosbare laag-moleculaire pektinefracties werden echter te lage waarden gevonden. Bevredigende resultaten voor het AGA-gehalte in deze preparaten werden verkregen met de mhdp-methode. De veresteringsgraad werd berekend uit het AGA-gehalte en het methanolgehalte.

In hoofdstuk 4 zijn de resultaten van het onderzoek weergegeven en geëvalueerd. Uit een chemische karakterisering bleek dat de celwandpolysachariden van abrikozen en abrikozennectar bestaan uit pektinestoffen, hemi-cellulose en cellulose, waarvan de pektinestoffen in de hoogste concentraties voorkomen. Serum, afgescheiden van abrikozennectar, bevatte hoofdzakelijk pektinestoffen met een hoge veresteringsgraad en had een laag gehalte aan neutrale suikers. De pulp-

fraktie van de nectar bevatte een hoog gehalte aan neutrale suikers, vooral arabinose en glucose. De veresteringsgraad van het pektine in de pulpfraktie bleek lager dan van het pektine in het serum

Onderzoek naar het troebelingsgedrag toonde aan dat zowel in gepasteuriseerde als in ongepasteuriseerde abrikozenectar de troebeling langzaam bezinkt en een gel gevormd wordt. In ongepasteuriseerde nectar bleek het serum een aanzienlijke hoeveelheid pectine te bevatten, desondanks was de serumviscositeit lager dan die van gepasteuriseerde nectar. Geconcludeerd werd dat dit toe te schrijven was aan de restactiviteit van enzymen in de grondstof. Door toevoeging van technische pectinasepreparaten kon de troebeling in meer of mindere mater gestabiliseerd worden. Enzympreparaten met alleen polygalacturonase-activiteit waren in staat de stabiliteit te verbeteren, terwijl preparaten die pektine-esterase, polygalacturonase, pektine lyase en ook hemicellulasen, cellulasen en proteasen bevatten en daardoor een breed werkingsspectrum hebben, de troebeling volledig konden stabiliseren. Door toevoeging van combinaties van zuivere pektolytische enzymen konden dezelfde resultaten verkregen worden.

Uit suspensieproeven bleek dat serumpektine geen rol speelt bij het stabiliseren van de troebeling: door afbraak van het serumpektine kon de troebelingsstabiliteit zelfs verbeterd worden. Afbraak van de pektinestoffen in het serum èn in de pulpdeeltjes verhoogde de troebelingsstabiliteit meer dan de afbraak van het serumpektine alleen.

Om het mechanisme van de stabilisering door technische pektinasepreparaten vast te stellen werden de veranderingen in de samenstelling van de celwanden en de afbraakpatronen van het serumpektine gevolgd. Hetzelfde werd ook gedaan voor de afzonderlijke enzymactiviteiten aanwezig in de technische preparaten. Pulpdeeltjes bleken gestabiliseerd te kunnen worden door de toevoeging van pektinelyase, polygalacturonase + schimmel pektine-esterase en ook door polygalacturonase + exo-arabinase. Pektine-esterase, protease en exo-arabinase afzonderlijk hadden noch invloed op de troebelingsstabiliteit noch op de gelvorming. Polygalacturonase kon gelvorming verhinderen en de troebelingsstabiliteit verbeteren.

De enzymsystemen die de troebeling konden stabiliseren waren in staat grotere hoeveelheden rhamnose, arabinose, galactose en AGA uit de pulpdeeltjes in oplossing te brengen dan de niet-stabiliserende enzymen. Microscopisch onderzoek toonde aan dat in stabiele nectars de cellen gefragmenteerd waren en slechts weinig intakte cellen waren overgebleven. Door stapsgewijze extractie met achtereenvolgens water en oplossingen van ammoniumoxalaat, zoutzuur en

natriumhydroxide werden verschillende pektinefracties uit AIS van abrikozen geïsoleerd. De wateroplosbare en de zoutzuur oplosbare fractie omvatten resp. 40.3% en 37.4% van het totaal aanwezige pectine. De zoutzuuroplosbare fractie bleek een hoog gehalte aan neutrale suikers te bevatten, vooral arabinose. De stabiliserende enzymen bleken deze pectinefractie goed te kunnen afbreken, de niet stabiliserende enzymen konden dit slechts gedeeltelijk. De enzymatische afbraak van de celwanden bleek samen te hangen met de mate waarin de enzymen het zoutzuuroplosbare pektine konden afbreken. Deze pectinefractie speelde bovendien een belangrijke rol bij de gelvorming en het klaren van arbikozenectar. Een vergelijking van commerciële en in het laboratorium bereide nectars liet zien dat fragmentatie van de cellen door een intensieve homogenisatie onvoldoende was om een stabiele troebeling te verkrijgen. Toevoeging van stabilisatoren, zoals aangegeven door de fabrikant, zijn noodzakelijk om de gewenste stabiliteit te verkrijgen. In het laboratorium was het mogelijk nectars te bereiden met een stabiele troebeling door behandeling met stabiliserende enzymen zonder intensieve mechanische homogenisatie.

Uit dichtheidsmetingen aan de pulpdeeltjes en uit reologische metingen aan abrikozenectar bleek dat de stabiliserende enzymen de dichtheid van de deeltjes meer verlaagden en de zwichtspanning meer verhoogden dan de niet stabiliserende enzymen.

In hoofdstuk 5 volgt een samenvattende discussie over het onderzoek en wordt een mechanisme voor de stabilisering van de troebeling in abrikozenectar voorgesteld.

List of abbreviations

AGA = anhydrogalacturonic acid
AIS = alcohol insoluble solids
Ara = arabinose
DE = degree of esterification
DEAE = diethylamino-ethyl
g = acceleration due to gravity ($m.s^{-2}$)
Gal = galactose
GalpA = galactopyranuronic acid
GLC = gas-liquid chromatography
Glu = glucose
HS = HCl-soluble pectin
M = molar concentration
MHDP = m-hydroxydiphenyl
OS = oxalate-soluble pectin
Pa = pascal ($N.m^{-2}$)
PG = polygalacturonase
PL = pectin lyase
Rha = rhamnose
WS = water-soluble pectin
Xyl = xylose

Summary

The objectives of this study were to investigate the effect of pectolytic enzymes on cloud stability of apricot nectar and to elucidate the mechanism of cloud stability by means of modern analytical techniques. The subject of this study is introduced in Chapter 1.

Chapter 2 consists of a review of the literature on aspects of cloud stability in fruit juices and nectars. Firstly, studies on pectic substances in relation to the structure of fruit pectin are surveyed and then studies on pectolytic enzymes are reviewed. The chemical composition and type of cloud particles in various fruit juices and nectars, particularly orange juice, are discussed. Cloud stability of fruit juices and nectars are then discussed in relation to the action of pectolytic enzymes which de-stabilize cloud particles in turbid apple juice and stabilize cloud particles in cloudy fruit nectars. Finally, studies on the effect of mechanical homogenization on the stability of cloudy fruit and vegetable preparations are reviewed.

The materials and methods used in this study are presented in Chapter 3. Special attention has been paid to the determination of the amount of anhydrogalacturonic acid (AGA) and degree of esterification (DE) in soluble pectin and insoluble pectin. The methods used are the copper-ion exchange method, and the colorimetric m-hydroxydiphenyl (MHDP) method combined with determination of methanol by using gas liquid chromatography (GLC). The copper method was found to be reliable and to produce reproducible results for the concentration of AGA in alcohol insoluble solids (AIS) of fruit tissue and to give low values for the soluble pectin fraction which contains low molecular weight fragments. In these preparations AGA was best determined by the MHDP method and DE calculated from AGA concentration and methanol concentration (analysed by GLC).

The results of this study are presented and discussed in Chapter 4. The cell wall polysaccharides of apricot fruit and nectar were composed of pectic polysaccharide, hemicellulose and cellulose. Pectic polysaccharide made up the greater part of the cell wall polysaccharides. The serum separated from apricot nectar was found to contain mainly galacturonides with a high DE and small amounts of neutral sugars, while cloud particles were found to be rich in neutral sugars, especially arabinose and glucose. The DE of the pectin of cloud

particles was lower than that of pectin in serum.

Cloud loss behaviour in apricot nectar is discussed in this chapter. In both pasteurized and unpasteurized apricot nectar, cloud particles settled slowly and a gel formed. However, in the unpasteurized nectar the serum was shown to contain a considerable amount of pectin but to be of lower viscosity than the serum of the pasteurized nectar. Thus it was concluded that this is due to residual enzyme activity in the raw material.

Addition of commercial pectolytic enzyme preparations stabilized cloud particles to a varying extent. Enzymes which contain only PG activity improved the level of stability whereas wide spectrum preparations containing activities of PE, PG, PL and also hemicellulase, cellulase and protease stabilized the cloud completely. The addition of purified pectolytic enzymes produced similar results.

Re-suspension experiments indicate that serum pectin is not essential for cloud stability but on the contrary the degradation of serum pectin improved cloud stability. Degradation of pectin of serum and cloud particles was shown to improve cloud stability more than did degradation of soluble pectin only.

To elucidate the cloud stability obtained by commercial pectolytic enzymes, changes in cell wall composition and the degradation pattern of serum pectin were monitored. This was also studied using individual enzymes present in commercial preparations. Cloud particles stabilized with the addition of PL, PG + mould PE, and also with PG + exo-arabinase. PE, protease, and exo-arabinase did not affect cloud stability or gel formation but PG prevented gel formation and improved the level of cloud stability.

The stabilizing enzyme systems solubilized larger amounts of rhamnose, arabinose, galactose and AGA from the cloud particles than did non-stabilizing enzymes. Further, examination under the microscope showed that in stable nectar, the cell walls were ruptured and only few intact cells remained. Sequential extraction of pectic substancesof apricot puree AIS with water, solutions of ammonium oxalate, acid, and alkali, revealed that the HCl-soluble pectin contained high amounts of neutral sugars, particularly arabinose. This pectin fraction could be degraded by the stabilizing enzymes but only partly degraded by non-stabilizing enzymes. Enzymatic destruction of the cell wall depends on the degree to which the enzyme degrades this pectin fraction. This pectin fraction also plays an important role in gel formation and clarification of apricot nectar. A comparison of commercial and laboratory-prepared nectars showed that intensive homogenization to rupture the cells, was insufficient to

obtain cloud stability. It was also necessary to use stabilizing agents as indicated by the manufacturers. Cloud stability was achieved in laboratory-prepared nectar with stabilizing enzymes without applying intensive mechanical homogenization.

The determination of cloud particle density and the characterization of flow properties of apricot nectar showed that the stabilizing enzymes decreased the cloud particle density and increased the yield stress in the nectar more than did the non-stabilizing enzymes.

In Chapter 5, the results of this study are discussed and a mechanism of cloud stability in apricot nectar is proposed.

Contents

1 Introduction

Fruit nectars are beverages having a high content of fruit ingredients, sugar and sometimes acid. The production of fruit nectars is of great importance in fruit juice industry. They are prepared from fruits which are not suitable for direct processing into juice, that is, fruits the colour pigment and flavour constituents of which are bound mainly to pulp particles, but which as pulpy or cloudy preparations are too thick to be drinkable (e.g., apricot and mango). Clear juices which are obtained by pressing but which are too acid to be consumed as such are also processed into nectars (e.g., black currant and sour cherry). Also, fruit juice having a very strong flavour (e.g., passion fruit) have to be diluted to make an attractive beverage. Finally, fruit nectar can be produced from more mature fruit which cannot be used satisfactorily in standard processing.

Nutritionally, cloudy fruit nectars contain higher amount of dietary fibers, β-carotene and other pulp-associated compounds than clear juices. Nectars are diluted fruit products, and therefore the concentration of water-soluble constituents, such as, minerals and soluble vitamins, is lower than in clear juices.

Commercially, mainly apricot and peach nectars are produced and also small amounts of pear, plum, and citrus nectars. Nectars are also prepared from tropical fruit, for example, mango, guava, passion fruit, papaya, and banana. Apricot nectar was selected as a model for this study because the raw material is readily available in cans.

According to the Codex Alimentarius (FAO, 1981), apricot nectar is "the unfermented but fermentable pulpy product, intended for direct consumption, obtained by blending the total edible part of sound and ripe apricots with water and sugars or honey and preserved by physical means. The nectar must contain at least 35% w/w fruit ingredients". The EEC directive number 75/726/EEC (Council Directive, 1975) specifies the amount of fruit ingredient to be not less than 40% (w/w).

Apricot nectar is a suspension of disintegrated apricot tissue (cloud particles) in an aqueous medium (serum). The essential problem in all cloudy beverages is cloud loss. When left to stand, the cloud particles tend to settle out leaving a clear supernatant layer. Because most of the colour pigments and aroma components are localized in the cloud particles, this separation is a serious quality defect which reduces the attractiveness and marketability of the product. Substantial efforts have been made by technologists and research workers to prevent cloud loss in fruit nectars. Attention has been focussed on the use of homogenization as a mechanical means to reduce the particle size (Robinson *et al.*, 1956; Bertuzzi, 1961; Bielig & Rouwen, 1975; Zeh, 1984).

The use of commercial pectolytic enzyme preparations has also been studied (Gierschner & Baumann, 1969; Grampp, 1969; Šulc & Vijičič, 1973; Strübi, 1976; Schmitt, 1980), and also both means (Weiss & Sämann, 1972). However, conclusions are based mainly on empirical results, and little is known about the structure of the cloud particles or about the relationship between cloud stability and the effect of pectolytic enzymes.

The objectives of the present study are:

- -to characterize cloud loss behaviour in apricot nectar;
- -to stabilize cloud by means of commercial pectolytic enzyme preparations;
- -to study changes in the structural features of cloud particles and the chemical composition of serum as a result of enzymatic treatment and to relate these changes to cloud stability;
- -to evaluate the effect of the individual activities present in the commercial pectolytic preparations in regard to cloud stability;
- -to characterize changes in the density of cloud particles and the flow behaviour of the nectar as a result of enzymatic treatment.

On the basis of this work, a theory of cloud stability is proposed and also a practical way to achieve cloud stability in apricot nectar is proposed.

2 Review of literature

2.1 PECTIC SUBSTANCES

Pectic substances are a group of acidic polysaccharides which are widely distributed in the tissues of all higher plants. They are present mainly in the middle lamella and the primary cell walls (Northcote, 1958) but rarely in the secondary cell walls. Several reviews on pectic substances have been published (Doesburg, 1965; McCready, 1970; Pilnik & Zwiker, 1970; Voragen & Pilnik, 1970; Fogarty & Kelly, 1983). Pectic substances are polygalacturonides with non-uronide carbohydrates covalently bound to an unbranched chain of (1→4)α-D-galacturonic acid units. The carboxyl groups of the galacturonic acids are partly esterified with methanol, and the remaining groups are present as salt. Some hydroxyl groups on C-2 and C-3 may be acetylated. The monomer possesses the C-1 conformation and consequently, the hydroxyl groups at C-1 and C-4 are in an axial position. Because of the presence of various neutral sugars, pectins are considered to be heteropolysaccharides. Rhamnose is considered to be an integral part of the main chain, thus forming a rhamnogalacturonan to which side chains consisting of arabinose, galactose, xylose, and glucose are linked (Aspinall *et al.*, 1970; Northcote, 1972; Talmadge *et al.*, 1973; Kato & Noguchi, 1976). Mannose, glucose, and apiose may also be present in some pectins but in small amounts. Kawabata & Sawayama (1975) from analyses of the sugar constituents of pectic substances extracted from 20 fruit species and four commercial pectins found that arabinose and galactose were present in the largest amount, followed by rhamnose and xylose. The proportion of total neutral sugars varied from 4% to 16% of the pectin fraction weight. Rombouts *et al.* (1983) found ferulic acid to be linked to some arabinose residues in the side chains in sugar beet pectin.

The structural features of pectic substances have been studied extensively (Barrett & Northcote, 1965; Talmadge *et al.*, 1973; Albersheim, 1975; Knee *et al.*, 1975). de Vries *et al.* (1982) showed that in apple pectins extracted under mild conditions and subjected to enzymatic degradation, more than 90% of the uronic acid residues were present as homogalacturonan (smooth regions), and neutral

sugar side chains located in repeating blocks which in total contain less than 5% of the uronic acid residues (hairy regions). Uronic acid residues in the hairy regions were shown to be fully esterified with methanol. de Vries *et al.* (1983) also showed that the hairy regions consisted of rhamnogalacturonan fragments carrying arabinogalactan and galactan chains and single unit xylose side groups. The same features were found in apple juice pectins by Rouau & Thibault (1984). Aspinall & Fanous (1984) found that apple pectins are also linked to highly branched pure arabinan (α-L-arabinofuranan) and proposed a model for apple pectin (see Fig. 1) which, together with the findings of de Vries *et al.* (1982, 1983), demonstrates the appearance of the hairy regions.

Pectic substances extracted from cherry fruit are also characterized by smooth and hairy regions (Thibault, 1983), and the neutral sugars are concentrated in less than 11% of the uronic acid residues. The hairy regions of cherry pectin contain both short and long side chains. Stevens & Selvendran (1984) stated that the rhamnogalacturonan in carrot is linked to highly branched arabinan and slightly branched galactans.

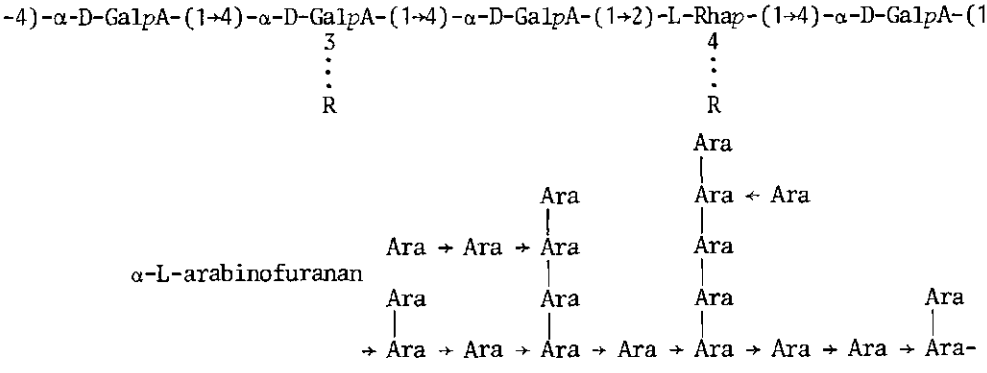


Fig. 1. α-D Galacturonan chains with occasional attachment of side chains, where R = α-L-arabinofuranan (Aspinall & Fanous, 1984).

2.2 PECTOLYTIC ENZYMES

Pectolytic enzymes are produced by micro-organisms and occur in higher plants. They play an important rôle in ripening, post-harvest changes, processing and storage of fruits and vegetables. Pectolytic enzymes have been reviewed by Fogarty & Ward (1974), Macmillan & Sheiman (1974), Rexová-Benková & Markovic

(1976), Rombouts & Pilnik (1980), Pilnik & Rombouts (1981) and Fogarty & Kelly (1983). These enzymes have been divided into two groups, saponifying enzymes (pectinesterase), and depolymerizing enzymes (hydrolases and lyases).

2.2.1 Pectinesterase (PE)

Pectinesterase (EC 3.1.1.11) is found in many higher plants and is also produced by mould, yeast, and bacteria. Various sources of PE are listed in Versteeg (1979). These enzymes have high specificity in hydrolysing the methyl esters of pectic acid. Mould PE cleaves the ester groups randomly in a manner resembling alkali and acid saponification (Ishii *et al.*, 1978; Baron *et al.*, 1980; Dongowski & Bock, 1981; Kohn *et al.*, 1983). Miller & Macmillan (1971) showed that 50% of the activity of *Fusarium oxysporum* pectinesterase occurs at the reducing end of highly esterified pectin chain and the remaining activity at other positions along the molecules. Plant PEs attack the pectin chain from the reducing end or next to free carboxyl groups and then proceed linearly along the molecules (Versteeg, 1979). Therefore, blocks of carboxyl groups are formed which impart the high sensitivity of pectin to calcium ions (Krop & Pilnik, 1974a; Kohn *at al.*, 1983). Plant PE cannot saponify mono-, di- and trigalacturonic acid methylesters (McCready & Seegmiller, 1954). Further, the enzymatic de-esterification of highly esterified pectin (degree of esterification (DE) 92%) by orange PE is impeded (Versteeg, 1979), perhaps because of obstruction of PE action by galactose side chains. It is not known whether this is also the case for mould PE. Ishii *et al.* (1978) demonstrated that purified PE from *Aspergillus japonicus* could reduce the DE of highly esterified pectin to 38% after 6 hr of enzyme action. Versteeg (1979) has demonstrated that some orange PEs are subject to end product inhibition and that the substrate affinity of orange PE increases with decreasing DE. In general, the optimum pH range for plant PE is 7 to 8 compared with 4 to 5 for mould PE.

2.2.2 Depolymerizing enzymes

These enzymes are differentiated according to:

- preference for pectin, pectic acid, or oligogalacturonates as substrate;
- the mode of action: hydrolysis or transeliminative mechanism;
- random (endo-) or terminal (exo-) attack.

The classification proposed by Pilnik *et al.* (1973) for this group of enzymes is set out in Table 1, and the structure of the substrate and the mode of action of various pectolytic enzymes is set out in Fig. 2.

Endo-polygalacturonases (PG). Endo-polygalacturonases are produced by numerous mould and bacteria and some yeasts, and are also frequently found in higher plants. They hydrolyse glycosidic bonds next to free carboxyl groups, thus causing a rapid reduction in the viscosity of the substrate. The best substrate is polygalacturonic acid, but pectins of different DE may also be attacked. The rate of hydrolysis of pectin by PG isolated from *Aspergillus niger* of different DE was found to decrease with increasing DE (Koller & Neukom, 1969). Pectin with a DE above 75% was not hydrolysed. Thus it was concluded that two free carboxyl groups at a certain distance apart are required to form binding sites for the enzyme molecule. However, in the cell wall of higher plants, PG can attack and solubilize pectins (protopectin) with a high DE, depending on the distribution of the carboxyl groups along the molecules. Because of this action, polygalacturonases are considered to participate in the softening of fruit tissue during ripening of some fruits, for example, tomatoes (Doesburg, 1965; Wallner & Bloom, 1977; Huber, 1983); peaches (Pressey *et al.*, 1971; Shewfelt *et al.*, 1971); pears (Ahmed & Labavitch, 1980; Bartley *et al.*, 1982); and strawberries (Woodward, 1972).

The end products of endo-PG action on pectic acid are mono-, di- and trigalacturonic acid (Koller & Neukom, 1969). Fogarty & Kelly (1983) distinguished two different mechanisms of attack by PG: single chain multiple attack which involves rapid formation of the end product; and multi-chain

Table 1. Proposed classification of depolymerizing pectolytic enzymes (Pilnik *et al.*, 1973).

Hydrolases	Lyases	
Polygalacturonase (PG)		Pectin Lyase (PL)
1. Endo-PG (EC 3.2.1.15)	3. Endo-Pectate Lyase (EC 4.2.2.2)	5. Endo-PL (EC 4.2.2.10)
2. Exo-PG (EC 3.2.1.67)	4. Exo-Pectate Lyase (EC 4.2.2.9)	

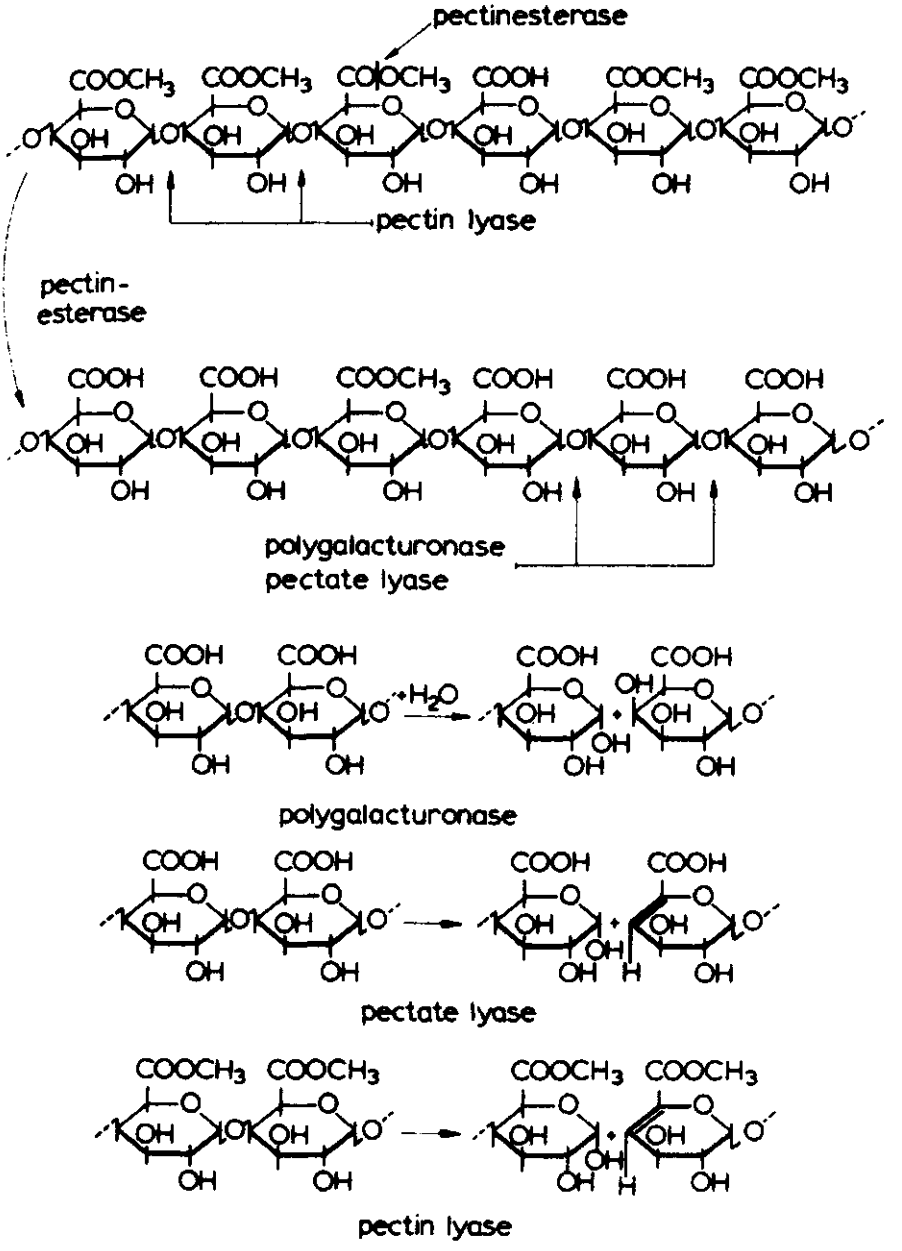


Fig. 2. Structure and points of attack of pectic substances by various pectolytic enzymes.

attack which means that mono-, di- and trigalacturonic acid accumulate as result of hydrolysis of higher oligogalacturonic acid.

Pectin lyase (PL). Albersheim *et al.* (1960) discovered that commercial pectinase preparations caused a trans-eliminative cleavage of the (1→4)α-glycosidic linkage in pectins, resulting in the formation of a double bond between C-4 and C-5 at the non-reducing end of one of the reaction products (Fig. 2). This enzyme which is called pectin lyase (PL) attacks, preferably at random, highly esterified pectin, which results in rapid reduction in viscosity, thus indicating an endo-attack. It splits the glycosidic bonds next to methylated carboxyl groups (β-elimination).

The activity of PL decreases rapidly with decreasing chain length. The smallest substrate for PL isolated from *Aspergillus niger* is trimethylgalacturonate (Voragen, 1972; Van Houdenhoven, 1975). PL is produced mainly by mould, but as yet has not been found in higher plants, and therefore is not considered to be involved in the ripening process of fruit. When added to fruit tissue, a strong maceration effect has been observed, for example, in potato (Keijbets, 1974; Ishii, 1978) and in onions (Ishii & Yokotsuka, 1971a).

2.3 COMPOSITION OF CLOUD PARTICLES IN FRUIT JUICES AND NECTARS

Cloud particles have been studied extensively in orange juice. As early as 1965, Scott *et al.* separated the particles present in orange juice into three fractions, pulp particles (coarse particles) which were separated at low centrifugal speed (600xg), and two other fractions of fine particles. These particles consist of 25% lipid and 75% material insoluble in alcohol, acetone, and hexane. The insoluble material consists of 80% pectin, 7% protein, and 1.5% phosphorus, in addition to about 2.5% of cellulose and hemicellulose. Scott *et al.* (1965) also analysed various structural tissues of orange fruit and found the cloud particle composition to be different. Thus, they concluded that cloud particles originate principally from the juice cells rather than from mechanical disintegration of the structural tissue. Baker & Bruemmer (1969) re-examined the insoluble material obtained by Scott *et al.* (1965) and found the proportion of pectin to be lower, 34%, and that of protein to be higher, 47%. They ascribed these differences to the methods used by Scott *et al.* (1965) to

estimate pectin and protein content. However, in both studies the pectin content was not based on the galacturonic acid content, but on the weight of the pectin which includes protein (Baker & Bruemmer, 1969) and probably other contaminating polysaccharides. Krop (1974) found that dried cloud particles of orange juice contain 8.8% anhydrogalacturonic acid and 32.6% protein. The DE of cloud particle pectin and serum pectin was 39.6% and 66.0%, respectively.

Mizrahi and Berk (1970) using light and electron microscopy to examine the cloud particles of freshly prepared Shamouti orange juice identified needle-like crystals of hesperidin (0.5 to 3.0 μm long, 0.05 to 2.0 μm thick), chromoplastides (1.0 μm diameter), amorphous (rag) particles (2.0 to 10.0 μm in size) and oil droplets (1 μm diameter). Krop (1974) also identified hesperidin crystals in the cloud particles of reconstituted orange juice (0.03 - 0.25 μm thick and 0.7 - 7.0 μm long). He determined the density of hesperidin isolated from the serum of Sunkist oranges and found it to be 1.23 $kg.m^{-3}$ and the density of pure hesperidin to be 1.45 $kg.m^{-3}$, the difference being attributed to impurities. Jewell (1975) provided support for this assumption by identifying oil droplets attached to the hesperidin crystals. Using light microscopy and electron scanning microscopy, he also identified cell walls, cellular and crystalline materials, and small droplets. He concluded that orange juice is a suspension of a complex mixture of particles derived from disintegrating fresh tissue.

In lemon juice, half of the cloud particles have been shown to be soluble in organic solvent (Bennett & Nelson, 1974). This soluble fraction was found to be a complex mixture, including fatty acid esters, sterols, phospholipids and flavonoid glycosides, and the remaining insoluble fraction to be composed largely of pectin and protein.

However, the amount of cloud particles and also the individual constituents have been found to vary with the maturity, variety and method of processing (Epstein & Mizrahi, 1975; Huggart *et al.*, 1975; Venolia & Peak, 1976; Royo Iranzo *et al.*, 1977).

As the cloud particles of fruit nectars are derived from disintegrated fruit tissue, study of their structure and composition requires a knowledge of the structure of the plant cell, particularly the cell wall. As will be seen from the following review, the nature of the cell wall largely determines the properties and texture of fruit and vegetable products. A model for the primary cell wall of suspension-cultured sycamore cells has been proposed by Keegstra *et al.* (1973) and modified by Robinson (1977) (Fig. 3). The cell wall is con-

sidered to be a network of discrete polysaccharides and proteins. The polysaccharides are pectic substances, hemicelluloses (matrix substances) and celluloses. Keegstra *et al.* (1973) have suggested that the matrix substances are interconnected by covalent bonds, but only non-covalent bonds are present between the xyloglucan (hemicellulose) and cellulose microfibrils. Xyloglucans are attached from the reducing end to the galactan side chains of the rhamnogalacturonan (pectin). Highly branched arabinogalactan acts as bridging molecule between the rhamnogalacturonan and hydroxyproline-rich protein. Knee (1975) found that protease or glycanase treatment of apple cell wall did not yield glycoprotein as may be expected from covalent linkages. Lamport & Epstein (1983) suggested that the primary cell wall is a truly woven structure of two concatenated polymers. Cellulose microfibrils (warp) penetrate the mesh of an extensin net (weft) suspended in a hydrophilic pectin and hemicellulose gel. More recently, McNeil *et al.* (1984) reported that the cell wall of higher plants is more complicated than that shown by the model of Keegstra *et al.* (1973) and also as modified by Robinson (1977).

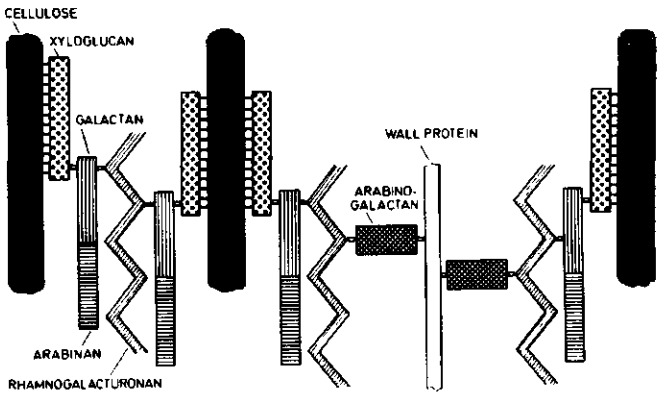


Fig. 3. Structure of the primary wall of suspension-cultured sycamore cell (Robinson (1977), modified from Keegstra *et al.* (1973)).

In higher plants, the cell walls of two neighbouring cells are connected by a cementing layer called middle lamella. This layer consists predominantly of pectic substances, as demonstrated by Albersheim & Killias (1963). The chemical composition of the cell wall is usually determined as the ethanol-insoluble

fraction, which consists primarily of the cell wall and middle lamella because the two elements are not easily differentiated. The polysaccharide and protein contents of cell wall material of various fruits and vegetables have been reported by Voragen *et al.* (1983); of apple, by Voragen *et al.* (1980); of carrot, by Kono & Yamazaki (1982); of pear, by Ahmed & Labavitch (1980); of tomato, by Brown & Stein (1977) and Pressey & Avant (1981); and of potato, by Le Tourneau (1956) and Hoff & Castro (1969).

The type and quantity of cloud particles are of direct consequence to cloud loss. In a study of the type of cloud particles in apple nectar, Strübi (1976) found that of the total particles, 57.8% consisted of individual cells and cell wall fragments, 17.5% of aggregates of two to five cells; 8.6% of aggregates of six to ten cells; and 16% of clusters containing more than ten cells. The cloud particles yielded 1.5% ethanol-insoluble residue which contained 22.4% pectin (DE 64.8%); and the remainder was protein, cellulose, and hemicellulose. In preparations made by Weiss & Sämann (1972), the cloud particles of apricot nectar were found to be composed of 85.7% individual cells, 5.2% broken cells, 3.7% cell wall fragments, 4.9% cell aggregates, and 3.7% vascular tissue fragments.

In general, the size of the cloud particle of fruit nectars is much higher than that given for orange juice. In a study of structure of laboratory-prepared and commercial apricot puree using a microscope, Reeve (1956) found that commercial apricot puree consisted of:

-intact, separate spherical cells (140 x 107 µm);
-oblong parenchyma cells (70 x 340 µm);
-clusters of three or more unseparated, intact parenchyma cells (130 x 340 µm);
-fragments of skin (30 x 60 x 160 µm);
-vascular tissue or rag (30 x 320 µm);
-crushed, broken cells and cell-wall fragments;
-cellular particles, fine granular matter of protoplasm and crystalloids freed from broken cells.

Three-quarters of the particles in each puree were found to be intact separate parenchyma cells, produced by solubilization of part of the middle lamella during blanching.

The suspended particles of commercial tomato juice were found to consist of separate intact cells, clusters of intact cells, broken cells and cell wall fragments, and skin vascular tissue fragment which accounted for 60%, 4%, 35% and 1% respectively of the total number of suspended solids (Reeve *et al.*, 1959).

2.4 CLOUD STABILITY OF CITRUS JUICES

About 70 years ago, cloud loss in orange juice was found to be due to the presence of enzymes in the juice (Cruess,1914) and that cloud particles could be maintained in suspension with adequate pasteurization.

In a review of cloud loss by Joslyn & Pilnik (1961), the oldest theory on this phenomenon is described as follows. Native orange PE de-esterifies soluble pectin, which acts as protective hydrocolloid in the juice. Then in the presence of calcium ions, the resultant low methoxyl pectin is precipitated as calcium pectate including the cloud particles. Primo Yufera *et al.* (1961) studied cloud loss phenomenon without taking into account serum pectin. Freshly prepared orange juice was separated into cloud particles and serum and then the cloud particles re-suspended in water. They found that during storage, insoluble pectin decreased and low methoxyl pectin increased. These changes were accompanied by an increase in sedimentation rate. Thus they concluded that clarification in orange juice depends on modifidation of the surface of cloud particles and not on alteration of serum pectin. Such modification was attributed to a native protopectinase which solubilizes part of pulp insoluble pectin, and pectineaterase which de-esterifies the solubilized pectin fraction and also the insoluble pectin. Primo Yufera *et al.* (1963) stained the pulp particles isolated from pasteurized cloud-stable juice and those from clarified juice with hydroxylamine ferric chloride. This colouring reagent is specific for methoxyl groups. Pulp particles of the cloud-stable juice were coloured deep red and those of the clarified juice light pink because of the loss of methoxyl groups. Baker & Bruemmer (1969) found that re-suspending cloud particles of unpasteurized fresh orange juice in water yielded a highly stable suspension, and thus concluded that soluble pectin is not required for cloud support. This is contrary to the early theory that soluble pectin acts as protective hydrocolloid to hold the cloud particles (Dietz & Rouse, 1953). Baker & Bruemmer (1969) also claimed that the serum pectin is a cloud de-stabilizing factor, especially when demethylated by PE and precipitated with calcium ions.

Mizrahi & Berk (1970) isolated cloud particles of freshly prepared orange juice and re-suspended them in synthetic sera containing calcium ions concentrations varying from 0 ppm to 500 ppm. They found that the cloud particles were negatively charged and that the addition of calcium ions did not affect cloud stability. They ascribed this to the extent of hydration of the cloud particles rather than to their electrical charge.

Krop & Pilnik (1974a) concluded that saponification of juice pectin is not sufficient to bring about cloud loss, calcium ions are required to accomplish clarification. Both serum pectin and cloud pectin are important in cloud loss (Krop, 1974) and differentiation between these pectin fractions is difficult. Krop (1974) formulated the mechanism of cloud loss in orange juice as follows. Native PE in orange saponifies both soluble and insoluble pectin. When a certain degree of saponification is achieved, the sensitivity of the pectin to calcium ions increases. Insoluble calcium pectate is formed and produces a sediment encompassing all cloud particles and hesperidin, thus leaving a clear supernatant.

Baker & Bruemmer (1969) were the first to use exogenous pectolytic enzymes to stabilize cloud in citrus juices. They separated fresh orange juice into serum and cloud particles, degraded serum pectin with a commercial pectolytic enzyme preparation (Klerzyme 200, Wallerstein Co., Morton Grove, USA) and then re-suspended the cloud particles. This resulted in a stable juice. Such juice which contained active enzyme was more stable than that which contained inactivated enzyme, because of the continuous degradation of pectin released from the cloud. According to Baker & Bruemmer (1969), cloud is destabilized by PE action on serum pectin. Because Klerzyme 200 had been used to clarify apple juice, they stated that the clarification mechanism in apple juice is different from that in orange juice.

The joint action of pectinase (Klerzyme 200) and protease (Ficin, Sigma Chemical, St. Louis, USA) gave better cloud stability than pectinase alone (Baker & Bruemmer, 1971). In another experiment, the addition of pectinase to unstrained orange juice increased the yield of strained juice, decreased the quantity of waste pulp, and reduced the gross viscosity. Thus, the enzymatic treatment not only degraded the soluble and insoluble pectin but also solubilized part of the cell walls of the particles. This effect may have contributed to the stability achieved. Baker & Bruemmer (1972) examined six other commercial pectolytic enzymes and found that one accelerated clarification while the other five stabilized cloud particles to a varying extent. Cloud stability was not achieved in freshly prepared orange juice by the addition of Biopectinase 700 (Biocon, Lexinton, KY, USA; Braddock, 1981), Irgazyme M-10 or Ultrazyme-100 (Novo Ferment, Basel, Switzerland; Chandler & Robertson, 1983). Baker & Bruemmer in 1972 concluded that the stabilization of cloud in orange juice by pectolytic enzymes is governed by the depolymerization of pectic substances to soluble pectates instead of to insoluble high polymer pectates, and that the selection of an appropriate pectinase to stabilize

natural cloud in orange juice depends on their depolymerizing activity on polygalacturonic acid and pectin.

Another approach to the study of cloud stabilization by enzymatic degradation of juice pectin is to use well characterized single activities. Krop & Pilnik (1974b) reported that the addition of yeast PG to PE-inactive orange juice had no effect on cloud loss, and when PG and PE acted simultaneously no cloud loss occurred. The addition of PL to PE-inactive juice prevented cloud loss in the juice when PE was added (Krop, 1974). However, PE-active juice was not a suitable substrate for PL because the juice pectin is demethylated, and therefore PG was 100 times more effective than PL. Possible pathways for enzymatic degradation of serum pectin in orange juice as summarized by Baker (1977) are set out in Fig. 4. Enzymatic destruction of either soluble pectin or low ester pectin is used to prevent the precipitation of low esterified pectin as pectate.

Krop & Pilnik (1974c) showed that commercial protease preparations have no positive effect on the cloud stability of orange juice. However, more recently Wobben & Tan (1983) patented a process to stabilize the cloud in citrus beverages by subjecting pasteurized concentrate or single strength juice to at least one enzyme having protease activity. They claimed that cloud stability depends on the degree of protein hydrolysis in the juice. At 75-95% hydrolysis ratio cloud stability was extended for several months. Further, they concluded that mechanical pretreatment (homogenization) or enzymatic pretreatment (hemicellulase and/or cellulase) is required to render the protein more accessible to protease. The pretreatments may be responsible for stabilization. Homogenization was found to improve cloud stability in orange juice by reducing the size of the pulp particles (Loeffler, 1941; Ahmed & Bhatti, 1971; Epstein & Mizrahi, 1975; Baker, 1977).

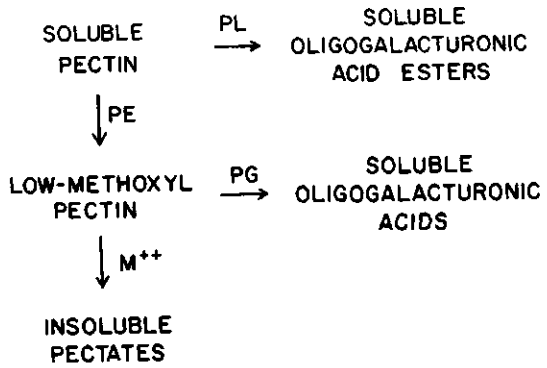


Fig. 4. Possible pathways for enzymatic degradation of serum pectin of orange juice (Baker, 1977).

2.5 CLOUD STABILITY OF NON-CITRUS JUICES AND NECTARS

Cloud stability must be maintained in cloudy fruit juices, such as tomato juice, and in cloudy fruit nectars, but cloud must be destabilized and removed in clear juices, such as, apple juice and grape juice.

2.5.1 Enzymatic clarification

In fruit juice technology, pectolytic enzymes were first used to clarify apple juice. After pressing the apple pulp, a viscous turbid juice is obtained. The soluble pectin in the juice is responsible for the high viscosity and for maintaining the fine particles in suspension. The addition of pectinase reduces the viscosity, and coagulates and precipitates cloud particles (Mehlitz, 1930). Yamazaki *et al.* (1964) studied the composition of the turbid material and formulated a hypothesis for the enzymatic clarification of apple juice. They found that cloud particles in apple juice consist of 36% protein and suggested that this is bound to polygalacturonides in which arabinose, galactose, glucose and galacturonic acid are the composite sugars. In the cloud particles, the positively charged protein is surrounded by a negatively charged layer of pectin which acts as a protective hydrocolloid to maintain the cloud particles in suspension (Fig. 5a). The mechanism of clarification by pectolytic enzymes proposed by Yamazaki *et al.* (1964) is set out in Fig. 5b. Partial or total removal of pectin exposes the positively charged protein of the particles which, together with the negative charge of the pectin of other particles, form a complex and settle out.

Endo (1965a) isolated and purified endo-PG and PE from a culture extract of *Coniothyrium diplodiela*. The addition of these enzymes separately to apple juice did not achieve clarification, but when mixed together the juice was clarified (Endo, 1965b). This he ascribed to the presence in the juice of

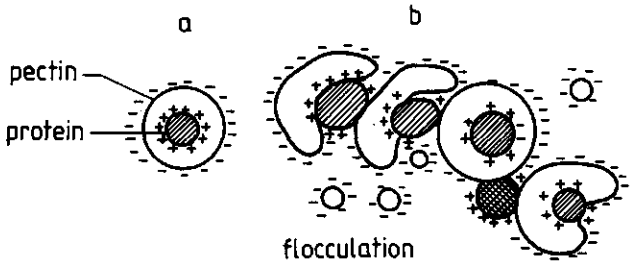


Fig. 5. Clarification mechanism in apple juice (Yamazaki *et al.*, 1964).

highly esterified soluble pectin (DE 90%), which must be degraded to insure clarification. He also found that the joint action of exo-PG and PE did not bring about clarification nor did the addition of cellulase, protease, or amylase. Thus he concluded that clarification of apple juice by the commercial enzyme preparation of *Coniothyrium diplodiela* is due to endo-PG and PE action. The clarification mechanism was explained in three stages (Endo, 1965c):

1. solubilization of insoluble pectin bound to the cloud particles;
2. decrease in viscosity of juice due to degradation of dissolved pectin;
3. flocculation of the suspended particles.

This was confirmed by Yamazaki *et al.* (1967) who reported that the combined action of endo-PG and PE of different origin was effective in clarifying apple juice. They concluded that the clarification mechanism occurred in two steps, destruction of the protective colloid (pectin), followed by electrostatic neutralization.

The fact that the destruction by PG and PE of highly esterified pectin in apple juice is necessary for clarification is substantiated by the fact that clarification was also achieved with PL from *Aspergillus sojae* (Ishii & Yokotsuka, 1971b and 1972). This effect was apparent even in the absence of proteases, hemicellulases and cellulases. Therefore, the mode of action of commercial pectinase containing PL activity on apple juice clarification is due to PL action (Ducroo, 1976). Eliminative splitting by PL is considered to be an advantage because it degrades pectic substances without the liberation of methanol (Ishii & Yokotsuka, 1972; Reymond & Bush, 1972).

Addition of PL was less effective in achieving clarification of grape juice than of apple juice, the degree of esterification being 33-68% and 93% respectively (Ishii & Yokotsuka, 1973). Ducroo (1976) reported that the native PE in grapes is responsible for the low degree of esterification. This explains the effectiveness of PG+PE in respect to grape juice clarification rather than the action of PL.

2.5.2 Enzymatic modification of fruit cell walls

Voragen & Pilnik (1981) have distinguished four stages in parenchymal cell wall degradation by pectolytic enzymes:

-*stage 1.* Maceration transforms organized tissue into a suspension of cells which should remain mostly intact. Some investigators have claimed that maceration is a viable process to prepare cloud stable nectar.

- *stage 2*. Pulp-enzyming is the process by which cell walls are partly destroyed and the dissolved pectin is largely degraded. In the German language this process has been given the misleading name of 'Maische Fermentation'. This process is used to improve the pressing characteristics of soft fruits (e.g., strawberries and black currants), and of fruits the pressing characteristics of which have deteriorated during storage (e.g. apple).
- *stage 3*. Liquefaction, which aims at complete dissolution of cell walls, is achieved by the addition of cellulolytic enzymes to preparations used for pulp-enzyming.
- *stage 4*. Saccharification, which is liquefaction to mainly monomer and dimer carbohydrates, occurs in the presence of hemicellulases and glycosidases in the liquefying enzyme preparations.

There are numerous enzyme preparations on the market which will bring about these modifications to fruit tissue. Their action, which is largely dependent on their individual activities (e.g. PE, PG and PL), characterizes them as being either macerating or pulp enzyming, Voragen & Pilnik (1981) used purified yeast PG, orange PE, mould PE and exo-glucanase to hydrolyse apple cell walls and to achieve selectively the four stages given above. Dongowski & Bock (1981) have described a method by which enzyme preparations can be characterized as either macerating or pulp-enzyming. They worked with purified mould PE and purified mould PG and used a suspension of apple cell wall material as substrate. With PG, water insoluble, high molecular weight, highly esterified, apple protopectin was tranformed into water soluble, high molecular weight pectin fraction, whereby the viscosity of the liquid phase of apple cell wall suspension is increased. This they described as macerating action. The viscosity is decreased again by depolymerization of the pectin if enough PE activity is present. The enzyme action is then changed from macerating to pulp-enzyming.

Ishii & Yokotsuka (1971a) showed that PL isolated from *Aspergillus sojae* macerated onion tissue rapidly into separate cells, but in potato, radish and cabbage tissue, maceration is gradual. They suggested that in higher plants highly esterified pectic substances are present in the middle lamella which is involved in cementing cell walls together. Ishii (1976) demonstrated that the middle lamella pectin of various plants differ particularly with regard to the degree of esterification. He found that onion tissue was more susceptible to PL action, and radish tissue was more susceptible to PG action.

However, not only pectinases but also other polysaccharide-degrading enzymes,

such as α-L-arabinofuranosidase, can macerate plant tissue (Gremli & Neukom, 1968). This enzyme was demonstrated to macerate cucumber tissue but not potato tissue. An endo-1,5-α-arabinase isolated from *Bacillus subtilis* was used to macerate potato tissue (Yosihara & Kaji, 1983). These findings indicate that hemicellulose rich in arabinan also participates in the coherence of plant tissue. Thus, it may be concluded that structural features of the plant cell wall determine whether a particular enzyme activity is macerating or pulp-enzyming.

Two commercially available enzyme preparations are used widely commercially and also in research as macerating enzymes, namely, Rohament P and Irgazyme M-10. They are relatively pure PG preparations (Grampp, 1969; Norde, 1969) and do not require isolation and purification.

Norde (1969) reported that apple suspensions prepared with Rohament P are more cloud stable than those prepared mechanically. The absence of enzymatic browning led him to conclude that the cell walls were intact. Grampp (1969) found that carrot and tomato tissues treated with Rohament P gave a pulpy juice with high yield, colour and cloud stability. The latter was attributed to the increase of water soluble pectin of high molecular weight.

The work of Sulč & Vujičič (1973) with various stone fruits and berries shows that Rohament P acts as macerating enzyme only if the degree of esterification of the fruit insoluble pectin is higher than 75%. The enzyme attacks the protopectin and solubilizes it as a high molecular weight fraction which causes an increase in serum viscosity. However, if the fruit is overripe or if endogenous PE is active, the degree of esterification of the fruit pectin decreases and the effect of Rohament P becomes pulp-enzyming or clarifying. They observed that when fruit nectar was prepared from macerated puree a cloud-stable product was obtained. This stability was ascribed to the high serum viscosity and to hydration capacity of high-esterified pectin present in the cell wall of the particles. Strübi (1976) used Irgazyme M-10 to macerate apple tissue and found that highly esterified pectin was released and low esterified pectin remained in the cell walls. He concluded that cloud stability is due to high serum viscosity and the hydration capacity of the soluble pectin which acts as protective hydrocolloid.

From the foregoing review on maceration by Rohament P and Irgazyme M-10, cloud stability may be considered to be related to the increase in serum viscosity and the hydration capacity of soluble pectin or cell wall pectin. However, results obtained from other studies indicate that this may not be the

case. According to Robinson *et al.* (1956), neither pectin in the suspended pulp particles nor pectin in the serum have a direct effect on the degree of settling in tomato juice. The more solids suspended in the juice, the less is the degree of settling (Bock *et al.*, 1973). Zetelaki-Horvath & Gatai (1977) applied a pure endo-PG from *Aspergillus awamori* to tomato juice. The untreated samples separated spontaneously into serum and a fibrous part, but samples treated with endo-PG were stable. Their results also indicate that viscosity of the treated samples was lower than that for the untreated samples, thus suggesting that pectin was degraded. More recently, in a study of the effect of pectolytic enzymes (Pectinex and Ultrazyme 100) and a cellulolytic enzyme (Onozuka R10, Yakult Biochemicals Co. Ltd., Japan) on the cloud stability of tomato juice, Shomer *et al.* (1984) showed that degradation of cell wall pectin, in which the cellulose microfibrils are embedded, permits partial dispersion of the microfibrils and induces swelling of the cell wall. Cellulase activity caused a drastic reduction in the suspeding properties because of partial or complete degradation of the microfibrils. These results also indicate that soluble pectin is not required for the stability of tomato juice. Gierschner & Baumann (1969) and Strübi (1976) found that the addition of Pectinex, which has the properties of a pulp-enzyming preparation, stabilized cloud particles in apple nectar, but Samsonova *et al.* (1979) and Hugo (1981) found that the macerating enzymes, Rohament P and Irgazyme M-10, did not stabilize cloud particles in apricot nectar.

Weiss & Sämann (1972) have shown that the addition of pectolytic enzyme preparations, either separately or in combination with homogenization, gave a cloud-stable apricot nectar, but homogenization alone failed to achieve cloud stability. The serum viscosity of the stable nectar was only half that of unstable nectar indicating that soluble pectin was degraded and was not necessary for cloud stability. They concluded that cloud stable nectar is obtained when the fruit particles are degraded to such an extent that 22±5% of cell wall fragments are formed.

As discussed for orange juice (Section 2.4), pectinase preparations with pulp-enzyming properties can achieve cloud stabilization (Baker & Bruemmer, 1971; Krop, 1974), indicating that serum viscosity is not required for this process. More recently, van Vliet & van Hooijdonk (1984) have shown that rheologically, orange juice can be considered to be a gel when left undisturbed and that the property of the gel structure is required to prevent suspended particles sedimenting. This gel is characterized by a yield stress which is due

primarily to pulp particles and not to serum polysaccharides or protein. Juice free from pulp particles was shown to behave like a Newtonian liquid. Results presented in this dissertation also show that the cloud particles of apricot nectar form a gel during sedimentation.

In this review, an attempt has been made to cover work on the use of enzymes for the preparation of nectar. In a recent description of manufacturing methods by MacKenzie *et al.* (1982) no mention was made of the use of enzyme even though the problem of cloud stability was discussed (Fig. 6). This is also true of previous descriptions of the manufacture of fruit nectar from apricots (Tressler, 1954; Luh *et al.*, 1959; Luh, 1980a; Hugo, 1981); peaches (Beavens & Beatti, 1942; Lee & Pederson, 1950); pears (Luh, 1980a); plums (Eddy & Veldhuis, 1941; Luh, 1980a); berries (Kuusi & Kiesvaara, 1968); and tropical fruits, such as, mango, guava, passion fruit, papaya and banana (Saeed *et al.*, 1975; Hugo, 1981; Luh, 1980b; Lambert, 1981).

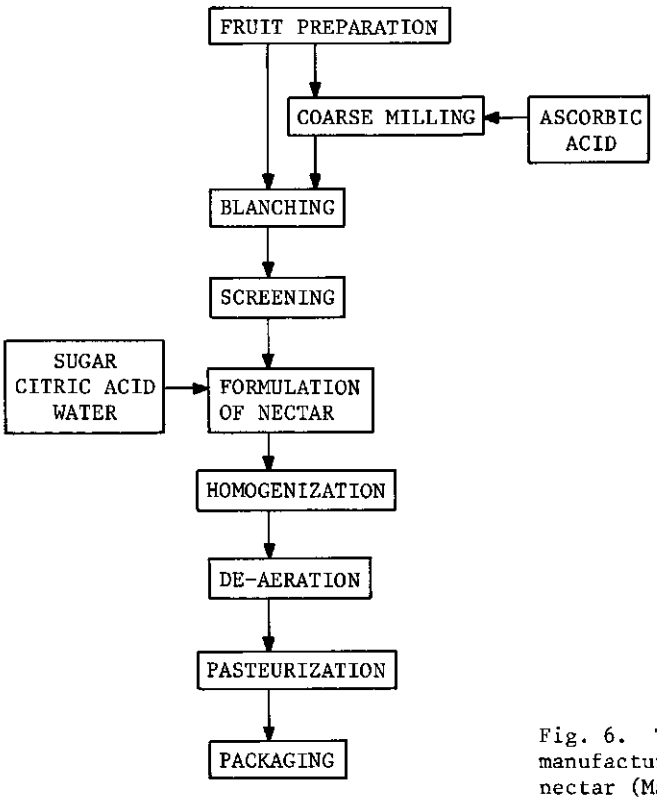


Fig. 6. The process for manufacture of pulpy fruit nectar (MacKenzie *et al.*, 1982).

2.6 MECHANICAL MACERATION (HOMOGENIZATION)

A factor which promotes cloud stability in fruit nectars is the particle size of the macerated fruit tissue, as shown by Stokes' law (Pilnik, 1968; Schobinger, 1980)

$$v = (\rho_s - \rho_c)\ g\ d^2/18\ \eta_c$$

where

v = sedimentation rate of the particles ($m.s^{-1}$)
ρ_s = density of the particles ($kg.m^{-3}$)
ρ_c = density of the continuous phase ($kg.m^{-3}$)
g = acceleration due to gravity ($m.s^{-2}$)
d = diameter of the particles (m)
η_c = viscosity of the continuous phase (Pa.s).

Mizrahi & Berk (1970) suggested that cloud particles which are less than 3 μm in diameter are subject to Brownian movement and do not sediment. From Stokes' law, it is apparent that the sedimentation rate is proportional to the square of the particle diameter. Therefore, the homogenization which reduces the particle size has an effect on the cloud stability of the nectar.

Homogenization should yield a product in which no sedimentation of cloud particles occurs during storage (Bertuzzi, 1961; Soavi Figli, 1980). The effect of homogenization on the viscosity and cloud stability of cloudy carrot juice and apricot nectar has been studied by Bielig & Rouwen (1975). They found that at a certain homogenization pressure, the viscosity of the juice increased. This increment was ascribed to disintegration of fruit tissue and liberation of pectin from the middle lamella of the cell wall. In the presence of sugar, acid or calcium ions, the solubilized pectin formed a gel, and the viscosity increased. At the same time, the pectin in the cloud particles formed a protective hydrocolloid layer which allowed particle density to be closer to that of serum, thus cloud-stable juice was obtained. However, no direct relationship was found between the intensity of homogenization and gross viscosity, serum viscosity and cloud stability. As a juice obtained from pulp treated with enzyme (Rohament P) was used, changes which occurred during homogenization may not have been entirely due to the homogenization process. Weiss & Sämann (1972) were not able to produce cloud-stable apricot nectar by homogenization alone.

Similar results were obtained by Strübi (1976) with apple nectar, and by Schmitt (1980) with carrot juice. In both studies enzyme treatment of fruit tissue gave better cloud stability than did homogenization.

3 Materials and methods

3.1 MATERIALS

Apricot fruit. Canned apricots of the varieties Oriellons and Bulida which are produced in Spain and Morocco were used. These were supplied by Hero, Breda, the Netherlands. Canned apricot were used to insure a continuous supply of fruit which cannot be done with fresh fruit. The industrial manufacture of apricot nectar is also based partly on canned fruit either as puree or as fruit halves, because of the short season for the supply of fresh fruit. Canned apricot puree supplied by Obipectin Ltd., Bischofszell, Switzerland, and fresh apricots from the local market were also used to prepare nectars. These were compared with nectar prepared from canned fruit halves in respect to cloud loss behaviour. As no differences were found in the nectar prepared from the three sources, only canned apricot halves were used.

sugar solution. Commercial-quality sucrose was used to prepare the sugar solution.

Pectolytic enzymes. Two types of enzymes were used to prepare nectars:

- -commercial pectolytic enzymes
  - ·Pectinex (Novo Ferment AG, Basel, Switzerland)
  - ·Pectinol C (Röhm, Darmstadt, Fed. Rep. of Germany)
  - ·Rapidase C-80 (Gist-Brocades, Delft, the Netherlands)
  - ·Rohament P (Röhm, Darmstadt, Fed. Rep. of Germany)
  - ·Ultrazyme 100 (Novo Ferment AG, Basel, Switzerland)
- -purified pectolytic enzymes prepared in the Department of Food Science, Agricultural University
  - ·endo-polygalacturonase (PG): purified yeast PG was obtained from *Kluyveromyces fragilis* and purified mould PG was isolated from Ultrazyme 20(Novo Ferment AG) as described by Vijayalakshmi *et al.*(1978);
  - .pectinesterase (PE): crude orange PE was produced using the method of Krop (1974), and orange PE and mould PE (Seclin, France) were

purified using the method described by Baron *et al.* (1980);
pectin lyase (PL): endo-PL isolated from Ultrazyme 20 was purified by adsorption and partition chromatography according to the method described by van Houdenhoven (1975).

Exo-arabinase (EC 3.2.1.55). Pectinase no. 29 (Gist-Brocades) which is a commercial pectolytic preparation containing high activity of arabinases was used to isolate exo-arabinase as described by Voragen *et al.* (1982).

Protease. Commercial protease preparation GGA, J 6470 (Ciba-Geigy, Basel, Switzerland) secreted in the culture media of *Aspergillus melleus* was used.

Chemicals. The chemicals used in this study were fine grade (pro analysi).

3.2 METHODS

3.2.1 Preparation of apricot nectar

Apricot nectar, both treated and untreated with enzymes, was prepared according to the scheme set out in Fig. 7. After apricot puree was prepared it was placed in a reaction vessel (Fig. 8) at 40°C for 30 min and stirred occasionally. A sample (untreated sample) was withdrawn just before the enzyme was added. After the addition of the enzyme, samples were withdrawn at specified times and heated immediately in Erlenmeyer flasks for 10 min in a boiling water bath to inactivated the enzyme. The untreated sample was also heated. The puree was cooled in the water bath at room temperature, and then diluted with sugar solution 1:1 (w/w) to a final Brix value of 13°. Sodium benzoate (1 g/litre nectar) was added as a preservative. The nectar was then homogenized with an Ultra 'Turax (PMT-Tamson type 18-10) homogenizer at full speed (20,000 rpm/min) for 25 sec. The nectar was de-aerated in a rotary film evaporator and pasteurized in serum bottles in a boiling water bath for 10 min. A sample of the prepared nectar was stored at 20°C to observe the cloud loss behaviour and the remainder was separated directly into cloud particles and serum by centrifugation in a refrigerated superspeed Sovall RC2-B centrifuge for 20 min at 49,000 x g at 0°C. The sediment was washed twice with water and then re-centrifuged. The wash water was added to the serum. Alcohol insoluble solids (AIS) were prepared from both fractions

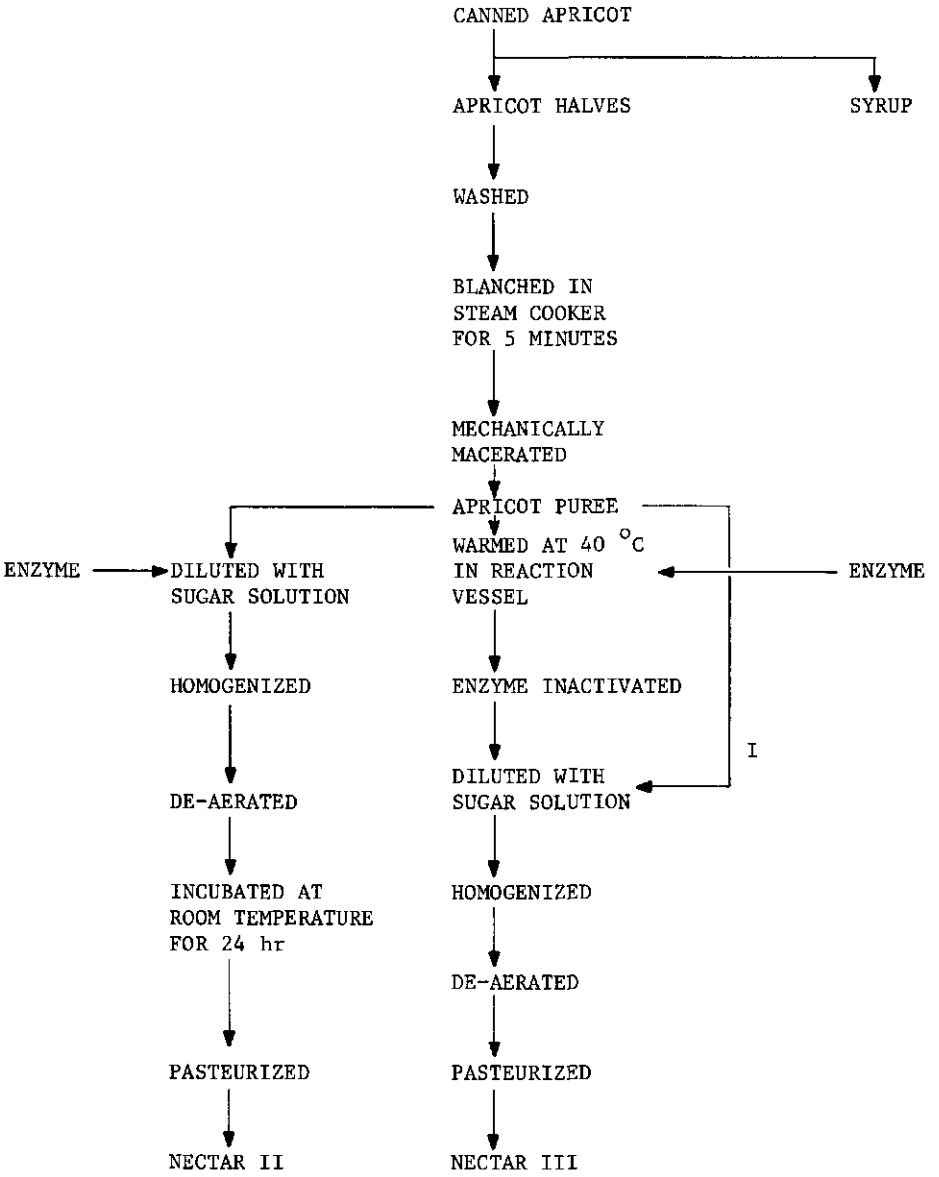


Fig. 7. Scheme for the preparation of apricot nectar.

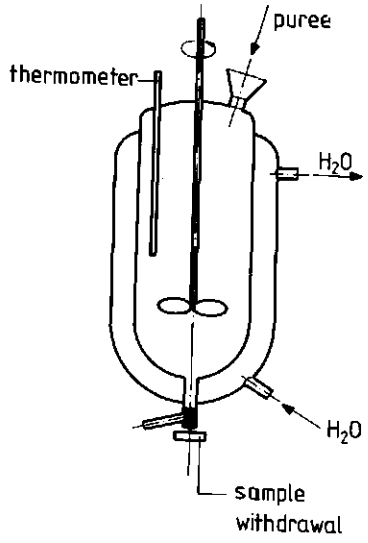


Fig. 8. Reaction vessel for enzymatic treatment of apricot puree.

3.2.2 Preparation of alcohol insoluble solids (AIS) from apricot puree and apricot cloud particles

Apricot puree was prepared as described in Fig. 7. The puree (1 kg) was collected directly from the finisher in an Erlenmeyer flask containing boiling ethanol (2 litres, 91% v/v) to inactivate the endogenous enzymes in the fruit. The mixture was left to stand at about 70 °C for 30 min and then filtered through a Büchner funnel. The particles were re-suspended in 1 litre ethanol (91% v/v) and extracted at room temperature for 1 hr with occasional stirring. This procedure was repeated four times until a clear extract was obtained. The particles were washed firstly with ethanol (96% v/v) and then with absolute acetone and air-dried. The dried residue was reduced to powder in a Culatti DFH 48 hammer-mill to particles smaller than 0.7 mm. AIS from cloud particles were prepared by re-suspending the particles in ethanol (91% v/v) and treated as described above.

3.2.3 Preparation of AIS from syrup and serum

Syrup (1 litre), that is the liquid part of the canned apricot, was mixed with 2 litres ethanol (91% v/v) and refrigerated overnight. The gel-like precipitate was collected on a cheese cloth and re-suspended in ethanol by means of an

Ultra Turax homogenizer. The precipitate was then collected on a cheese cloth. This procedure was repeated four times. The alcohol precipitate of serum of apricot nectar was separated by centrifuging at 16,000xg for 20 min in a refrigerated superspeed centrifuge (Sorvall RC2-B) instead of filtration. This was done because, some sera, in particular enzyme-treated sera, formed a sandy precipitate with alcohol which was difficult to filter. The residue was dissolved in appropriate minimum amount of distilled water, freeze dried and powdered as described previously.

3.2.4 Cloud stability measurements

Two methods were evaluated in order to find a suitable method to measure the cloud stability of apricot nectar. The first was the centrifugation method which is commonly used to measure the cloud stability of citrus juices. This involved centrifugation of a 10 ml sample in a conical centrifuge tube for 10 min at 360xg, and monitoring the turbidity of the supernatant by measuring the extinction (E) at 660 nm (Krop, 1974) in a spectrophotometer or colorimeter. The second method was the observation method. Nectar was placed in a 100 ml serum bottle and allowed to stand undisturbed at 20 ^{o}C. The height of the cloudy phase during sedimentation was recorded daily in the first week and every two or three days thereafter. The degree of stability was measured as a percentage of the height in respect to the total height of the nectar (100 ml), expressed as follows:

$$\text{degree of stability} = \frac{\text{height of cloudy phase}}{\text{total height}} \times 100$$

Results obtained with both methods for cloud-stable and cloud-unstable nectar are presented in Fig. 9. When centrifuged a much lower extinction value of the supernatant was obtained for stable than unstable nectar. Krop (1974) investigated various methods of measurement to determine the cloud stability of orange juice and also found the same phenomenon. Therefore in this present study, the observation method was selected to determine the cloud stability of apricot nectar. This is a time-consuming process but is a realistic method because it does not involve centrifugation. The formation of gel during cloud loss was observed by carefully tilting a serum bottle containing nectar to observe whether the gel is destroyed and mixed with the serum.

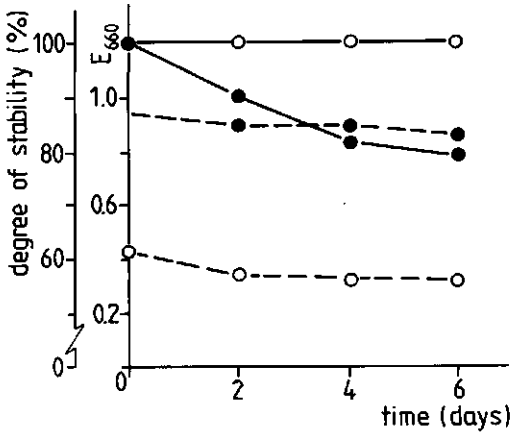


Fig. 9. Cloud stability of unstable (●) and stable (o) apricot nectar as measured by centrifugation method (---) and observation method (—).

3.2.5 Fractionation of AIS of apricot puree

AIS of apricot puree was fractionated into pectin, hemicellulose, and cellulose fractions as described by Voragen *et al.* (1983). Because of their important role, pectic substances were fractionated according to their solubility in water, in ammonium oxalate, in 0.05 N HCl and in 0.05 N NaOH (Fig. 10). Each fractionation was carried out by extracting three times with the extractant and then twice with distilled water. After each extraction, the mixture was centrifuged in a refrigerated superspeed centrifuge (Sorvall RC2-B) for 20 min at 16,000xg at 0 °C. The supernatants were dialysed against distilled water for 5 days and freeze-dried.

3.2.6 Enzyme Assays

Polygalacturonase (PG) activity was assayed colorimetrically by determining the increase in reducing groups by the Nelson-Somogyi method as described by Spiro (1966). Polygalacturonic acid (ICN Pharmaceuticals, Inc., Cleveland, Ohio, USA) was used as substrate. A mixture consisting of two parts of 0.2 M sodium succinate buffer pH 5.2, one part of polygalacturonic acid solution 1% (w/v), and one part of distilled water was subjected to the enzyme action and incubated at 30 °C for a specified period of time. One unit of PG is the amount

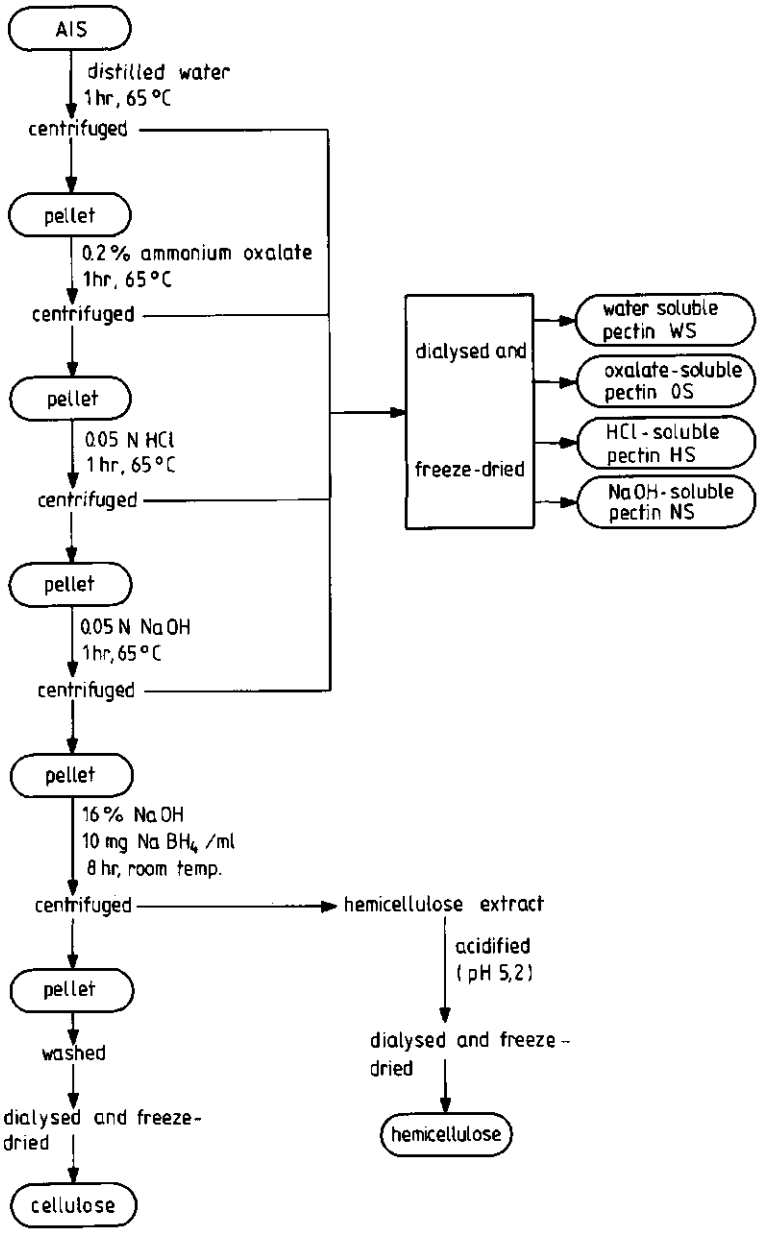


Fig. 10. Extraction of polysaccharide fractions of AIS of apricot puree.

of enzyme which catalyses the release of 1 μmole of reducing sugar per minute using glucose as standard.

Pectinesterase (PE) activity was determined titrimetrically according to the method of Vas *et al.* (1967) by estimating the free carboxyl groups formed in pectin as a result of enzyme action. The amount of 0.01 N NaOH required to maintain the pH of the substrate solution at 7.0 for orange PE or at 4.0 for mould PE at 30 °C, was measured using an automatic titrator (Combi Titrator 3D, Metroham AG, Herisau, Switzerland). The enzyme substrate was commercial apple pectin (Obipectin, green ribbon, DE 62.0%) solution 0.5% (w/v) in 0.15 M NaCl. One unit of PE is the amount of enzyme which liberates 1 μmole of carboxyl groups per minute.

Pectin lyase (PL) activity was determined by monitoring the increase in absorption at 235 nm of a solution of high methoxyl pectin (Voragen, 1972). Enzyme substrate consisting of 0.25 ml 1% (w/v) pectin solution (esterified pectin, DE 92.3%), 1.5 ml McIlvaine buffer pH 6.0 and 1.15 ml distilled water was pipetted into 10 mm quartz cuvette and placed in a water bath at 30 °C. The cuvette was placed in a thermostated chamber in the spectrophotometer (Zeiss, model PMQ II) and 0.1 ml of enzyme solution added. The increase in absorption at 235 nm was recorded with a Servogor recorder. One unit of PL activity is the amount of enzyme which increases the absorbance at 235 nm at the rate of 1 per minute.

Total activity of commercial pectolytic preparations refers to the overall effect of various pectolytic enzymes (e.g., PG, PE, and PL) present in a pectinase preparation on the viscosity of a solution of high methoxyl pectin. This was determined by monitoring the decrease in the viscosity of the pectin solution as a function of the reaction time; 9.5 ml of a 0.5% (w/v) solution of high methoxyl pectin (DE 89%) in 0.01 M sodium tartrate buffer pH 3.6 was pipetted into an Ubbelohde glass capillary viscometer at 30 °C. The flow time of the buffer at 30 °C was 27.3 sec. Enzyme solution (0.5 ml) was injected into the viscosemeter and mixed by passing air bubbles through it before measuring the flow time. The specific viscosity (η_{sp}) was calculated according to the following equation

$$\eta_{sp} = \frac{t - t_o}{t_o}$$

where

t_o = flow time of buffer

t = flow time of the reaction mixture.

The reciprocal of specific viscosity ($\frac{1}{\eta_{sp}}$) was plotted against the reaction time. The amount of enzyme which reduces the specific viscosity by 50% was calculated and the total activity was determined from the slope of the straight line ($\frac{1}{\eta_{sp}}$ vs time).

Exo-arabinase activity was determined colorimetrically by the Nelson-Somogyi method as described by Spiro (1966). Ultrafiltrate retentate isolated from apple juice obtained by liquefying apple pulp with pectolytic and cellulolytic enzymes (Voragen *et al.*, 1985) was used as substrate. The reaction mixture consisted of 50 µl enzyme source in 50 mM sodium acetate buffer pH 5.0, 100 µl 0.5% ultrafiltrate retentate in water and 350 µl sodium acetate buffer pH 5.0. After incubating for 1 hr at 30 °C, the increase in reducing groups was determined using arabinose as standard. One unit of arabinase activity is the amount of arabinase which catalyses the release of 1 µmole of reducing groups from ultrafiltrate retentate per minute.

3.2.7 Enzyme treatment of AIS of apricot puree

Portions of AIS residue (1 g) were weighed into seven 100 ml glass stoppered Erlenmeyer flasks and suspended in 60 ml 0.025 M sodium acetate buffer pH 3.6. The Erlenmeyer flasks were incubated in a Gallenkamp orbital incubator at 40 °C. When this temperature was reached, the enzyme solution was added and after 5, 15, 30, 60, 120 and 180 min, a flask was taken from the incubator. One flask was also removed just before the addition of enzyme (untreated). The contents of each flask were centrifuged in a refrigerated centrifuge for 20 min at 49,000xg at 0 °C. The supernatant was collected and the pellets washed twice with distilled water. The washing water and the supernatant were united, freeze-dried, and powdered. The pellets were also freeze-dried and powdered.

3.2.8 Enzymatic degradation of HCl-soluble pectin

Aliquots (50 mg) of the freeze-dried pectin preparation (see Fig. 10) were mixed with 5 ml distilled water using a magnetic stirrer at room temperature and then heated to boiling and allowed to dissolve completely. After the pectin solution was cooled to room temperature, the pH was raised to 3.6 by the addition of 1% tri-sodium citrate solution and diluted with distilled water to 8 ml.

After the addition of enzyme solution, the mixture was incubated at 40 °C overnight with occasional stirring. Finally the solution volume was adjusted to 10 ml, and 1 ml of the mixture was applied to gel permeation chromatography. The following types of enzymes and units of activity were added:

- -endo-PG (8 units)
- -endo-PL (4 units)
- -exo-arabinase (2 units)
- -endo-PG (8 units) + mould PE (5 units)
- -endo-PG (8 units) + exo-arabinase (2 units)
- -endo-PG (8 units) + mould PE (5 units) + exo-arabinase (2 units)
- -endo-PL (4 units) + exo-arabinase (2 units)
- -Pectinex was purified from contaminating sugars by dialysing 1 ml Pectinex against distilled water overnight at about 5 °C. An amount of dialysed solution equal to 0.25 ml of the original preparation was added to the pectin preparation.

3.2.9 Gel permeation chromatography

The extent of degradation of serum pectin was studied by gel permeation chromatography over a Sephacryl S-200 column (53 x 1.2 cm) with 0.05 M phosphate buffer pH 7.0 being used as the eluant at a flow rate of 0.35 ml/min. Pectin (5 mg) was applied to the column in 1 ml distilled water. The extent of degradation of enzyme-treated HCl-soluble pectin was studied in a similar way, except that a Sephacryl S-300 column (68 x 1.05 cm) was used and that 1 ml of solution containing 5 mg AGA was applied to the column. Experiments were performed at room temperature.

3.2.10 Ion-exchange chromatography

Freeze-dried pectin preparations (50 mg) from the various fractions (Fig. 10) were dissolved in 5 ml 0.01 M tris-succinate buffer pH 4.8 and applied to a DEAE cellulose column (11.5 x 2.2 cm). A linear gradient of tris-succinate buffer (from 0.01 M to 0.3 M) was used to elute the pectin from the column. After eluting with 3 litre gradient buffer, 300 ml 0.5 M tris-succinate buffer pH 4.8 followed by 300 ml 0.1 N NaOH was used to elute the pectin still bound to the column. Experiments were performed at 20 ^{o}C.

3.2.11 Microscopic examination of cloud particles

Nectar (1 ml) was diluted with water to 10 ml, shaken well and examined using a light microscope (Axiomat-Zeiss) fitted with a camera. The cells or/and cell clusters representing most of the cloud particles were photographed and the photographs produced represent the cells magnified 210 times.

3.2.12 Determination of the density of cloud particles

Density gradient centrifugation was used to determine the density of various cloud particles. A sucrose gradient from 40% to 65% (w/w) in 5% steps was layered sequentially in a centrifuge tube (length 97.5 mm, diameter 31.8 mm). After pipetting in the 65% sucrose layer, the solution for the subsequent steps in the gradient were layered in turn on top of the previous layer using a syringe as shown in Fig. 11a. Nectar (10 ml) was layered on top of the gradient which was then centrifuged for 3 hr at a mean centrifugal force of 42,000xg at 2 ^{o}C using a MSE superspeed-65 ultracentrifuge. The cloud particles sedimented to positions in the gradient according to their density and were collected as shown in Fig. 11b. The fractions were dialysed for three days against distilled water to which sodium azide was added as preservative and then freeze-dried and weighed.

3.2.13 Flow behaviour of apricot nectar

The flow behaviour of the nectar under constant stress was measured using Deer rheometer model PDR 81 with a concentric cylinder at 20 ^{o}C. The rotating parts in this rheometer are held by a frictionless air-bearing support. The

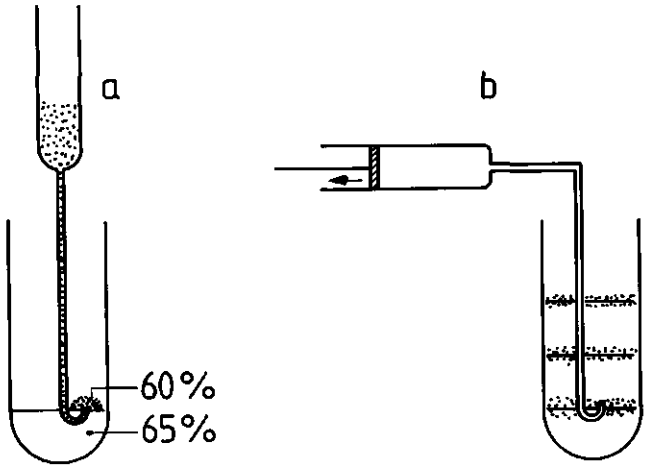


Fig. 11.a. Formation of gradients for density gradient centrifugation;
b. Withdrawal of fractions after centrifugation.

stress applied could be varied between 10^{-2} to 10 Pa and the shear rate from zero to 5000 sec^{-1}. The nectar was well mixed before being poured into the cylinder. The rheometer used was particularly suitable for measuring flow behaviour of apricot nectar because its measuring range permits the determination of the yield stress. The shear stress at which flow started was estimated as the yield stress.

The relative viscosity of serum was measured with an Ubbelohde glass capillary viscometer at 30 ^{o}C using the flow time of water as reference.

3.2.14 Analytical methods

Anhydrogalacturonic acid (AGA) *concentration and degree of esterification* (DE) of pectin were determined by two methods: the copper ion exchange method (Keijbets & Pilnik, 1974), and the colorimetric method by meta-hydroxydiphenyl (MHDP) as described by Ahmed & Labavitch (1977) combined with the determination of methanol by gas chromatography (MHDP + GLC method) as described by Versteeg (1979).

The copper ion exchange method (copper method) is based on the high affinity of pectin carboxylic acid groups for copper-ions. The pectin was precipitated as copper pectate and then washed to remove the free copper-ions. The bound copper-ions were released by washing with 0.6 N HCl. The copper-ions released were

determined using an atomic absorption spectrophotometer (Perkin-Elmer, model 2380) and their concentrations were calculated from a calibration curve made with standard solutions of copper. The procedure was repeated after alkali saponification in alcohol suspension of pectin.

AGA and DE were calculated from the amounts of copper-ions bound before and after saponification as shown below:

$$\% \text{ AGA} = \frac{\text{mg Cu-bound after saponification} \times 2 \times 176 \times 100}{63.6 \times \text{mg weighed sample}}$$

$$\text{DE} = [1 - (\text{mg Cu-bound before saponification/mg Cu-bound after saponification})] \times 100$$

With the MHDP + GLC method, the AGA was determined colorimetrically by MHDP. The methyl ester groups were hydrolysed by alkaline saponification and the resultant methanol converted to methyl nitrite which was determined by gas chromatography by means of head space sampling. The method of derivatization and the conditions of GLC column were similar to those described by Versteeg (1979), but a longer column (183 cm) and a higher column temperature (160 ^{o}C) was used. From the μ mole of methanol and μ mole of AGA, the DE was calculated:

$$\text{DE} = \frac{\text{Me} \times 0.032 \times 176}{\text{AGA}} \times 100\%$$

Me = concentration of methanol (mg/litre)
AGA = concentration of AGA (mg/litre)

During this study, the copper method was found to be useful for determining AGA concentration and DE in AIS of cloud particles and also of apricot puree, but not in extensively degraded serum pectin. Experience with the MHDP + GLC method was the reverse. Therefore, it was necessary to compare the results obtained with both methods. Three sera of apricot nectar with pectins of different molecular weight were used to determine the AGA concentration and DE by both methods. Serum A was untreated (high molecular weight), serum B was treated by endo-PG (intermediate molecular weight) and serum C was treated by PL (low molecular weight). With the copper method, the pectins in serum A and B were precipitated with copper sulphate, while that in serum C remained completely dissolved. Thus, this method could not

be applied with serum C. The results presented in Table 2 show that both methods give similar AGA concentration and DE for serum A, but for serum B the copper method gave lower values. This indicates that part of the pectin is probably too low in molecular weight to precipitate with copper-ions. In analysis of AIS of cloud particles and of puree, the copper method yielded higher DE values than MHDP + GLC method, but both methods yielded similar AGA concentrations.

Table 2. AGA concentration (as weight % of serum AIS) and DE of serum pectin of apricot nectar as determined by MHDP + GLC method and copper method

Serum	AGA concentration (%)		DE (%)	
	MHDP + GLC method	Copper method	MHDP + GLC method	Copper method
A - untreated	59.6	59.2	69.6	70.4
B - treated with PG	53.8	46.7	68.8	66.2
C - treated with PL	43.6	-	44.0	-

Similar results have been obtained by Voragen *et al.* (1983). Litchmann & Upton (1972) stated that for the determination of methanol, the pectin must be completely soluble in water. This indicates that for AIS preparations of fruit tissue, the insolubility of pectin in water is the main reason for the lower DE value obtained with the MHDP + GLC method. Thus it is reasonable to conclude that the copper method is reliable and that reproducible results are obtained with it for AIS of fruit tissue and also for soluble pectin which does not contain low molecular weight fragments. The MHDP + GLC method can be used for either intact or degraded soluble pectin.

The concentration of AGA in the fractions collected from gel permeation and ion-exchange chromatography was determined by the automated MHDP method (Thibault, 1979).

Sugar analysis. The neutral sugar composition was determined by GLC after acid hydrolysis and conversion to alditol acetates according to the method described by Jones & Albersheim (1972). This was carried out using 2 N trifluoroacetic acid (TFA) with inositol as internal standard. For the determination of neutral sugars of apricot puree AIS, cloud particle AIS, hemicellulose, and cellulose, the sample was digested with an enzyme mixture before hydrolysis with TFA to increase the recovery of sugars. The enzyme mixture was prepared from

de-saccharified Ultrazyme 100 and Maxazyme CL 2000. Ultrazyme 100 was de-saccharified by ultrafiltration. The enzyme was dissolved in 0.01 M sodium acetate buffer pH 5.2 and transferred into an ultrafiltration cell (Amicon Diaflo type 50) equipped with a YM 5 Diaflo filter. Maxazyme CL 2000 was de-saccharified by gel filtration on a Bio-gel, P-10 column. The enzyme mixture used to digest the sample contained the following activities:

- endo-glucanase (EC 3.2.1.4): 0.53 units/ml (on Akzo carboxymethyl cellulose AF 0305);
- exo-glucanase (EC 3.2.1.21): 0.01 units/ml (on Avicel cellulose, type SF Serva Feinbiochemica);
- Cellobiase (EC 3.2.1.91): 0.09 units/ml (on Difco cellobiose);
- endo-PG: 3.57 units/ml (on polygalacturonic acid);
- PE: 0.21 units/ml (on apple pectin, DE 62.0%).

Samples (3 mg) were weighed into reaction tubes and suspended in 1 ml of enzyme mixture. The tubes were incubated at 40 ^{o}C for 48 hr with occasional stirring. Subsequently, the water was removed under a stream of air at room temperature and hydrolyses of the sample was completed with 2 N TFA prior to the formation of the alditol derivates. These were separated on a gas chromatograph (Hewlett Packard, model 5750G) fitted with a dual flame ionization detector and a column (length, 300 cm; I.D. 3 mm) packed with 3.7 g of 3% OV 275 on Chromosorb W.AW 80-100 mesh. The temperature of the injection port was 250°C, of the flame detector 250°C, and of the oven 190°C. The gas chromatograph was connected to a LDC computer which calculated the neutral sugar concentration directly. The neutral sugars were calculated with the correction being made for the water taken up during the hydrolysis of the polysaccharide.

The amount of neutral sugars in the eluates from the gel or DEAE chromatography was monitored colorimetrically using phenol sulphuric acid (Dubois *et. al.*, 1956).

Cellulose content of the cloud particle AIS was determined according to the method described by Updegraff (1969).

Protein content was determined by the semi-automated micro-Kjeldahl method according to Roozen & Ouwehand (1978).

4 Results and dicussion

4.1 CHEMICAL COMPOSITION OF CANNED APRICOT FRUIT AND NECTAR

4.1.1 Canned apricot fruit

The raw material used in this study was apricot halves canned in water. As a result of diffusion of soluble material from the fruit, the Brix value of the liquid was about 4.5^{o}. It has therefore been referred to as syrup. Alcohol insoluble solids (AIS) were prepared from both apricot and syrup and analysed chemically. The chemical composition of AIS of the apricot halves is presented in Table 3. Neutral sugars and anhydrogalacturonic acid (AGA) constituted almost 80% of the AIS preparation, and the predominant neutral sugars were glucose and arabinose.

During the canning process (heat treatment) and storage of the canned apricots, components are solubilized in the fruit tissue and move to the liquid phase (syrup). The sugar composition of the AIS prepared from the syrup is presented in Table 4. The amount of AIS obtained from 100 ml syrup was 375.4 mg. The AIS consisted mainly of galacturonides of which 76.2% was esterified. This indicates that the material released into the syrup during processing is mainly pectic polysaccharides with a high DE. Navarro *et al.* (1982) studied the pectic polysaccharides of apricot fruit (var. Bulida) canned in syrups of various levels of acidity and found that the pectic substances released into syrup had the highest DE value. They also found that the pectic fraction solubilized in acidic medium was more methylated than soluble pectin of fresh fruit.

4.1.2 Apricot nectar

Apricot nectar was prepared as described in Section 3.2.1 and separated into cloud particles and serum. The chemical composition of the AIS of the nectar and of serum is given in Table 5. The AIS of cloud particles contained higher amounts of AGA and protein than that of the serum.

Table 3. Chemical composition of AIS prepared from canned apricots (expressed as proportion of weight of AIS (%))

Moisture	Protein	Starch	AGA	DE (%)	Neutral sugars					
					Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
8.7	8.0	0.2	25.9	63.5	1.2	9.3	4.6	1.8	4.5	22.6

Table 4. Sugar composition of AIS prepared from syrup of canned apricots (expressed as proportion of weight of AIS (%))

AGA	DE (%)	Neutral sugars					
		Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
61.7	76.2	0.8	2.8	0.5	t	2.8	4.5

t = traces

The DE of serum pectin was higher than that of cloud pectin, being 72.2% and 63.5% respectively.

Table 5. Chemical composition of AIS of apricot nectar and of its serum (All weights are expressed as mg/100 ml of nectar and serum, respectively.)

Constituent	Nectar	Serum
AGA	231.0	71.0
DE (%)	68.1	72.2
Protein	80.6	14.5
Cations		
Na	1.5	1.4
K	80.0	75.5
Ca	22.2	18.9
Mg	3.4	3.2

4.1.3 Polysaccharide composition of apricot puree and nectar

As apricot nectar is derived from the fruit puree, the composition of the puree, especially that of the polysaccharide constituents plays an important rôle in determining the composition of the nectar and its physical and chemical characteristics. Therefore, the various constituents of the puree need to be identified. AIS of the puree was prepared and fractionated into pectin, hemicellulose, and cellulose as described in Section 3.2.5. The amount of AIS obtained from 100 g puree was 2.1 g of which pectin, hemicellulose, and cellulose composed 52.0%, 14.7%, and 19.4%, respectively (Table 6). Pectic polysaccharides (rhamnose, arabinose, galactose, and AGA) form most of the cell wall polysaccharides (60%). The predominant sugar in the pectin fraction was AGA, and arabinose was the main neutral sugar. The hemicellulose fraction was rich in glucose, xylose, galactose and mannose, and considerable amounts of AGA, arabinose and rhamnose were also present. The sugar composition of hemicellulose fraction of morello cherries (Voragen *et al.*, 1983) is similar to that of apricot fruit, and both cherry morellos (*Prunus cerasus* L.) and apricots (*Prunus armeniaca* L.) are of the same genus. As expected, the cellulose fraction consisted mainly of glucose but contained also small amounts of AGA and arabinose.

Table 6. Sugar composition of AIS* of apricot nectar and the pectin, hemicellulose, and cellulose fractions (Sugar composition is expressed as mole %.)

Component	AIS	Pectin	Hemicellulose	Cellulose
Rhamnose	2.4	1.9	4.7	0.3
Arabinose	15.8	20.1	8.3	3.3
Xylose	5.2	0.6	18.8	1.4
Mannose	3.3	---	12.2	---
Galactose	6.4	3.5	17.0	0.3
Glucose	31.5	1.0	30.3	91.5
AGA	35.4	73.1	8.6	3.2

*AIS consists of 52.0%, 14.7% and 19.4% pectin, hemicellulose, and cellulose, respectively

The sugar composition of AIS of cloud particles and of serum of apricot nectar is given in Table 7. The chief constituent of the serum AIS was AGA (85.9%) and small amounts of arabinose and galactose were also present. AIS of cloud particles was composed of neutral sugars predominantly, arabinose and glucose, and AGA.

Table 7. Sugar composition of AIS of cloud particles and serum of apricot nectars (sugar composition expresses as mole %.)

Component	Cloud particles	Serum
Rhamnose	3.4	1.8
Arabinose	13.8	4.9
Xylose	5.0	1.3
Mannose	3.8	0.3
Galactose	6.6	3.4
Glucose	36.1	2.3
AGA	31.3	85.9

4.2 CLOUD LOSS PHENOMENON IN APRICOT NECTAR

Apricot nectar was kept in a graduated serum bottle and left to stand undisturbed for specified periods of time. When the cloud particles began to sediment they formed a gel, which, when left to stand for a longer period, contracted and a clear serum formed on the top of the gel. This clear serum may be considered to be partly syneresis water. This was also observed to varying extent with most commercial apricot nectars. Clarification and gel formation of cloudy passion fruit nectar has also been reported by Rensen (1984). However, the cloud loss behaviour of apricot nectar was quite distinct from that which occurs in orange juice, in which layers containing different shapes and sizes of particles form during clarification.

4.2.1 Changes in chemical composition during cloud loss in pasteurized and unpasteurized apricot nectar

During the preparation of nectar according to the method outlined in Section 3.2.1 it was noted that some of the canned apricots were soft. This softening can be attributed either to the activity of pectolytic enzymes from contaminating moulds, or to acid hydrolysis of the protopectin of fruit tissue. No difference in cloud behaviour was observed in pasteurized nectar prepared from either hard or soft fruit. However, because of the relevance to commercial preparation of nectars, the relationship was investigated between cloud loss phenomenon and any residual pectolytic activity which may have survived the heat treatment of the canning process. Pasteurized and unpasteurized apricot nectars were prepared from softened apricots and left undisturbed for a period of 18 days. Changes in chemical composition of AIS of the serum and of the cloud particles, and also changes in the serum viscosity were monitored.

Changes in the weight of AIS, the amount of AGA, and DE of cloud pectin and serum pectin of unpasteurized and pasteurized nectars both prepared from pasteurized apricot halves are set out in Table 8. In unpasteurized nectar, the amount of cloud particle AIS decreased and the amount of serum AIS increased. The amount of AGA in the cloud particle AIS decreased and that in the serum AIS increased. This suggests that in unpasteurized nectar a considerable amount of pectin is solubilized from the cloud particles to the serum when the nectar is left undisturbed. Solubilization did not occur in the pasteurized nectar. The amount of AIS in the cloud particles and in the serum remained unchanged

Table 8. Changes in the amount of AIS, and AGA and in DE of cloud particle pectin and serum pectin of pasteurized and unpasteurized apricot nectar during storage at 20 °C. (All weights are expressed as mg/100 ml nectar.)

Storage period (days)	Cloud particles			Serum		
	AIS	AGA	DE (%)	AIS	AGA	DE(%)
Unpasteurized apricot nectar						
0	652.1	167.6	68.3	156.1	98.7	72.0
6	620.3	133.5	60.6	194.5	136.3	72.2
12	591.2	116.1	60.1	210.1	149.4	70.1
18	572.4	105.0	55.9	231.2	159.1	70.5
Pasteurized apricot nectar						
0	643.8	160.5	67.7	165.2	102.5	73.1
18	645.1	162.1	68.3	166.0	105.1	72.2

when the nectar was left undisturbed (Table 8).

Changes in the amount of neutral sugars and AGA in cloud particle AIS of unpasteurized apricot nectar during 18 days storage at 20 °C are set out in Table 9. After 18 days the amount of rhamnose and arabinose had decreased

Table 9. Changes in the amount of sugars in the AIS of cloud particles of unpasteurized apricot nectar after storage for 18 days at 20 °C (All weights are expressed as mg/100 ml nectar.)

Component	Storage period (days)			
	0	6	12	18
Rhamnose	8.8	8.6	8.5	7.8
Arabinose	68.3	65.5	66.2	63.1
Xylose	27.4	26.9	27.1	27.2
Mannose	16.5	16.6	16.3	16.5
Galactose	34.4	33.9	33.8	33.6
Glucose	159.4	161.8	162.2	161.9
AGA	167.0	133.0	116.0	105.0

slightly, indicating that mainly rhamnogalacturonan with small amount of side chains had been solubilized during storage.

Serum viscosity in unpasteurized nectar decreased sharply, but in pasteurized nectar it remained constant (Fig. 12).

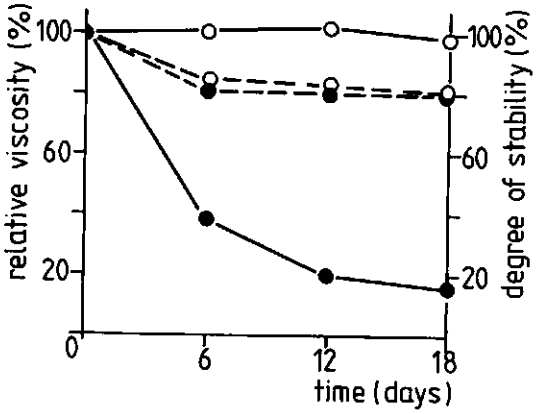


Fig. 12. Changes in serum viscosity (——) of pasteurized (o) and unpasteurized (•) apricot nectar and the cloud stability (---) during storage at 20 °C.

Cloud loss was similar in both pasteurized and unpasteurized nectar, but in the pasteurized nectar the serum was turbid for the first five days, while in the unpasteurized nectar, it was clear. This difference in turbidity is due to the higher viscosity of the pasteurized serum which allows the fine particles to be kept in suspension. The changes in the chemical composition and also in serum viscosity of pasteurized and unpasteurized nectars suggest that a specific native pectolytic enzyme is present. The action of this enzyme solubilizes the pectin from the cloud particles and also depolymerizes serum pectin which leads to a reduction in serum viscosity, but is not sufficient to make it alcohol soluble.

To identify this pectolytic activity, apricot puree was extracted with 1.25 M NaCl solution and the extract treated with a series of sequentially increasing concentrations of ammonium sulphate to precipitate the enzyme protein. After dialysis of the precipitate against distilled water, activities of PG, PE, and PL were assayed. No activity could be detected. In a second experiment, apricot puree was washed firstly with 0.2% sodium bisulphite and extracted with 0.2 M NaCl at pH 5.0. The extract was concentrated by ultrafiltration and then dialysed against 0.2 M NaCl and assayed for the presence of exo-PG and endo-PG activity. No activity was detected.

Softening in canned apricots has been studied by many investigators some of

whom have suggested that solubilization of protopectin from the canned fruit is due to the nature of the protopectin (Souty & Jacquemin, 1976). Navarro *et al.* (1982) concluded that most of the protopectin was solubilized because of the action of heat and especially of acidity. Other investigators have suggested that softening of canned apricot tissue is due to heat-stable enzymes from mould.

Attempts to recover active enzyme from canned apricot have been unsuccessful (Harper *et al.*, 1972; Harries & Dennis, 1980). In the present experiment evidence of the presence of pectolytic enzyme activity has been found, but it does not seem to play a rôle in preventing or inducing cloud loss of apricot nectar.

4.3 EFFECT OF COMMERCIAL PECTOLYTIC ENZYMES ON CLOUD STABILITY

To investigate the effect of pectolytic enzymes on the cloud stability of nectar, five commercial pectolytic enzyme preparations were added to apricot nectar. The enzyme and the concentrations added were as follows:

- -Pectinex, 0.5% (v/v);
- -Pectinol C, 0.025% (w/v);
- -Rohament P, 0.025% (w/v);
- -Rapidase C-80, 0.025% (w/v);
- -Ultrazyme 100, 0.010% (w/v).

Pectinex is a liquid preparation. Each of the other enzymes, which are powder preparations, was dissolved in 5 ml distilled water and added to the nectar as described in Section 3.2.1 (Nectar I Fig. 7). The effect of each of these enzyme preparations on cloud stability of apricot nectar is shown in Fig. 13. All preparations improved cloud stability to a varying extent. Pectinex-treated nectar remained stable throughout the experimental period of eight days and for several months afterwards. Nectars treated with Rapidase C-80 and Ultrazyme 100 were more cloud stable than those treated with Rohament P and Pectinol C. Nectar treated with Pectinol C clarified rapidly leaving a clear serum, and nectar treated with Rohament P for the first two days a turbid serum formed on the top which subsequently became clear. This effect is due mainly to the relatively higher serum viscosity of Rohament P treated nectar (η_{sp} 0.213) than of Pectinol C treated nectar (η_{sp} 0.092).

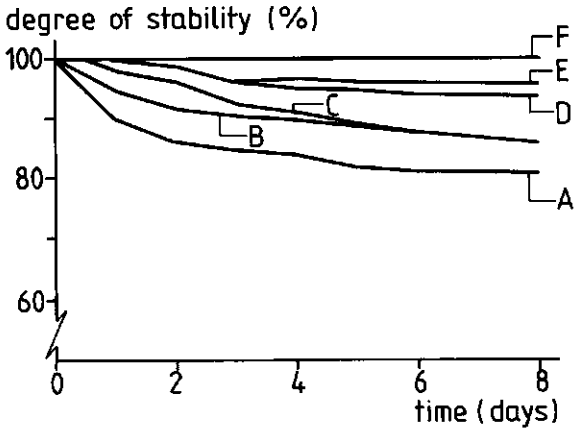


Fig. 13. Effect of commercial pectolytic enzymes on cloud stability of apricot nectar: A. Untreated; B. Pectinol C; C. Rohament P; D. Ultrazyme 100; E. Rapidase C-80; F. Pectinex.

To investigate these differences, activities of PE, PG, and PL and total overall pectolytic activity in each enzyme preparation were assayed (Table 10). The enzymes in descending order of total activity are Rapidase C-80, Ultrazyme 100, Pectinex, Pectinol C, and Rohament P. High total activity values coincide with high activities of PE, PG, and PL. Activities of PE and PL of Rapidase C-80 were higher than Ultrazyme 100, while PG activity of Ultrazyme 100 was higher than those of Rapidase C-80. Activities of PE and PL of Pectinol C were higher than those of Pectinex and there was little difference in their PG activity. On the other hand, Pectinex reduced the viscosity of the pectin solution (DE 89%) faster than did Pectinol C. Pectinex achieved cloud-stable nectar but Pectinol C did not.

Table 10. PE, PG, and PL activities and total activity* of pectolytic enzyme preparations (expressed as unit/g enzyme preparation and unit/ml Pectinex.)

Enzyme preparation	PE	PG	PL	Total activity
Pectinex	44.5	210.0	15.7	18.5
Pectinol C	118.2	182.0	33.0	11.9
Rohament P	trace	1727.0	trace	---
Rapidase C-80	765.0	423.4	86.8	117.7
Ultrazyme 100	125.0	694.0	74.8	67.4

* Total activity measured viscometrically by 0.5% high-esterified apple pectin solution (DE 89%).

Rohament P contains mainly PG activity and trace activities of PL and PE. The decrease in the viscosity of the 0.5% pectin solution (DE 89%) was very low. This can be explained by the fact that the suitable substrate for PG action is a low-methoxyl pectin. Similarly, Rohament P showed limited degradation of serum pectin (DE 72%) of apricot nectar.

When the amount of each enzyme added to the nectar was calculated on the basis of the activities of PE, PG, PL, and of total activity (Table 11), the highest activities were present in the Pectinex treatment. It is questionable whether the stabilizing effect of Pectinex is due to the high activity of the various pectolytic enzymes (PE, PG, and PL). If this were the case, other pectolytic enzyme preparations would also have stabilized nectar cloud particles when added in the same activity level as present in Pectinex. This is shown in Fig. 14. The amounts added were: Pectinex, 0.5% (v/v); Rapidase C-80, 0.1% (w/v); and Ultrazyme 100, 0.1% (w/v). All were found to have a stabilizing effect on nectar cloud particles but Pectinex was the most effective. However, this difference may be due to difficulty in obtaining the same activity spectrum with the other two enzyme preparations. The stabilizing effect of these enzyme preparations is probably a function of the combined action of PE, PG, and PL, and perhaps also of other enzyme activities, such as, proteases, cellulases, hemicellulases, and amylases, which are known to be present in such preparations. Therefore, an experiment was carried out in which purified pectolytic enzymes were added to apricot nectar singly and in combination with protease to investigate the stabilizing effect of Pectinex on cloud particles. Amounts of these enzymes were added to obtain the same level of activity as present in 0.5 ml of Pectinex (Table 10).

Table 11. PE, PG and PL activities and total activity of pectolytic enzyme preparations added to 100 ml of nectar (expressed in units added to 100 ml nectar).

Enzyme preparation	PE	PG	PL	Total activity
Pectinex	22.3	105.0	7.8	9.3
Pectinol C	2.1	4.6	0.8	0.3
Rohament P	trace	43.2	trace	---
Rapidase C-80	19.8	10.6	2.2	2.9
Ultrazyme 100	1.3	6.9	0.8	0.7

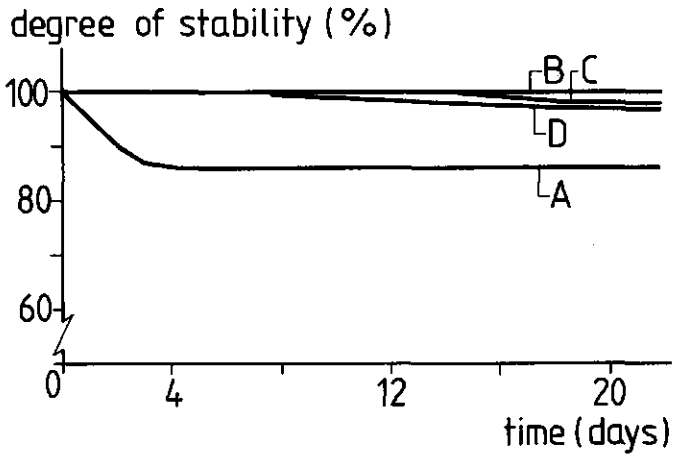


Fig. 14. Effect of Pectinex (B), and Rapidase C-80 (C) and Ultrazyme 100 (D) adjusted to give the same level of total activity as Pectinex on cloud stability of apricot nectar. (A) Untreated.

Protease (80 mg/100 ml nectar) was added alone and also together with PL. PL+protease and yeast PG+mould PE were shown to stabilize cloud particles (Fig. 15). PL alone also had a stabilizing effect for about 10 days, after that period a very small amount of clear serum formed. When added singly, protease, PG, and PE did not produce cloud-stable nectar. Thus it may be concluded that the stabilizing action of Pectinex is due to PG+PE and to PL action. The greater cloud stability achieved with the combination PL + protease may be explained by the fact that protease breaks down the cell wall protein, which is

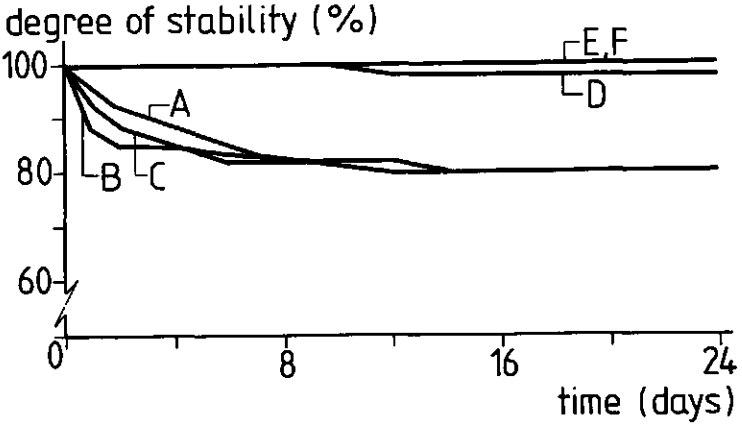


Fig. 15. Effect of purified pectolytic enzymes and protease on the cloud stability of apricot nectar: A. Untreated; B. Protease; C. Yeast-PG; D. PL; E. PL+Protease; F. Yeast-PG +mould PE.

known to be a structure element of plant cell walls, as shown in the model of Fig. 3. Degradation of this protein may render the pectin easily degradable by PL.

4.4 ROLE OF SERUM PECTIN AND CLOUD PECTIN ON CLOUD STABILITY

Cloud particles of apricot nectar were separated and re-suspended in normal serum and depectinized serum as shown in the scheme in Fig. 16. Nectar in which cloud particles were re-suspended in normal serum (nectar C, Fig. 17) was unstable throughout the experimental period. In nectar treated with PL (nectar B) both serum pectin and cloud pectin were degraded and the nectar was stable throughout the experimental period. When the cloud particles were re-suspended in depectinized serum (nectar D), the nectar remained stable for four days after which cloud stability decreased. In this nectar only the serum pectin was depolymerized. The decrease in cloud stability after four days may be attributed to the insoluble pectin in the cloud particles. This also suggests that degradation of the soluble pectin may improve cloud stability in the nectar.

In a further experiment, apricot nectar without sugar was treated with PL (nectar I) and the cloud particles separated and re-suspended by means of

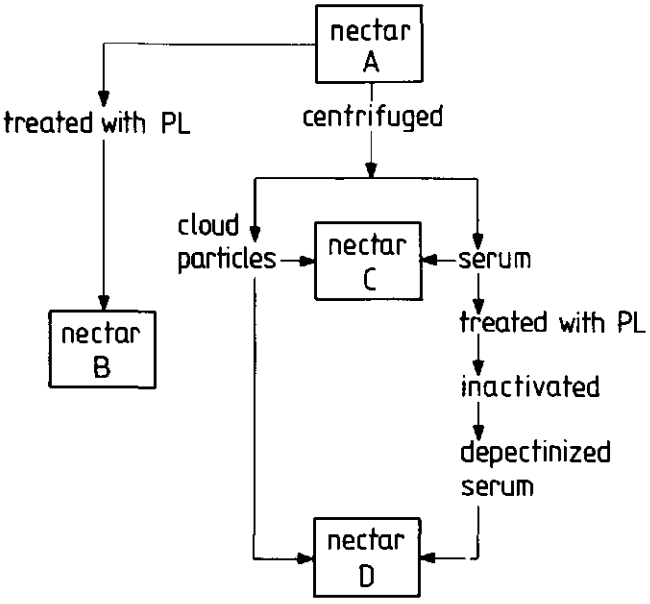


Fig. 16. Scheme of the re-suspension experiment of depectinized serum.

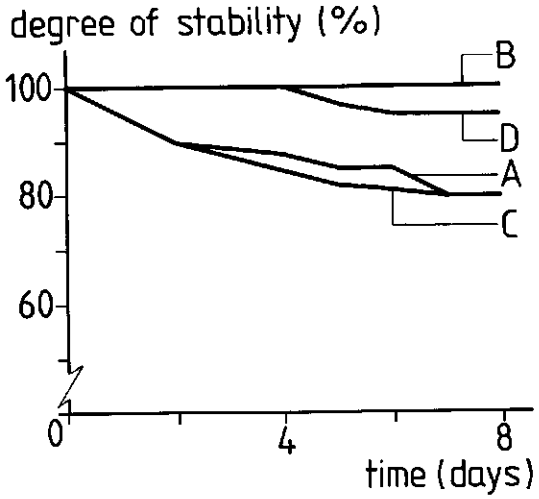


Fig. 17. Rôle of serum pectin in the cloud stability of apricot nectar. (For explanation of the letters, see Fig. 16).

vibrating mixer in the following solutions:

- the serum from which the cloud particles were isolated and sucrose to a final Brix value of 13° (nectar II);
- sugar solution, final Brix value 13° (nectar III);
- sugar solution and serum pectin, final Brix value 13° (nectar IV);
- serum pectin (nectar V).

Serum pectin was obtained by centrifuging untreated nectar and precipitating with alcohol. The precipitate was dried and powdered as described in Section 3.2.3. Fig. 18 shows that cloud particles were stable when re-suspended in

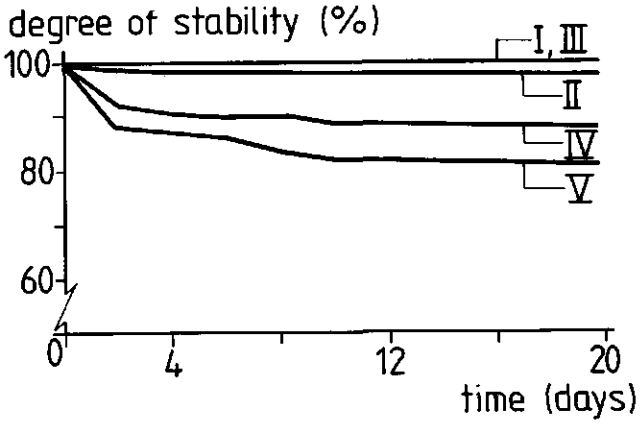


Fig. 18. Effect of re-suspending cloud particles of apricot nectar treated with PL in different sera on the cloud stability. (For explanation of the numbers, see the text.)

sugar solution and also in sugar solution + original serum, but after resuspension in a solution of serum pectin only or in a solution of serum pectin and sugar, the cloud particles settled out (Fig. 18). Thus it may be concluded that serum pectin was not necessary for cloud stability of apricot nectar. This finding is in agreement with results obtained for tomato juice (Shomer *et al.*, 1984); tomato-carrot macerates (Bock *et al.*, 1973); orange juice (Baker & Bruemmer, 1969; Krop, 1974); and apricot nectar (Weiss & Sämann, 1972). These re-suspension experiments are difficult to interpretate, but they prove the point that serum pectin is not necessary.

4.5 EFFECT OF PECTOLYTIC ENZYMES ON CHANGES IN THE CHEMICAL COMPOSITION OF APRICOT NECTAR

The results presented in Section 4.3 indicate that certain commercial pectolytic enzyme preparations and purified enzymes may have a stabilizing effect on cloud particles in apricot nectar. To investigate the mechanism involved, changes in cloud particles and serum brought about by these enzymes are need to be characterized. Thus in this section, changes in cell wall polysaccharides and serum are discussed. The method of enzymatic treatment of apricot cell wall material is described in Section 3.2.7. The activities of purified pectolytic enzymes (PE and PL) chosen were similar to those present in 0.25 ml Pectinex (Table 10). Rohament P was added to give the same activity of PG as that present in 0.25 ml Pectinex. The cloud stability measurements were made on nectar III in Fig. 7.

4.5.1 Effect of purified mould PE

The addition of purified mould PE to AIS of apricot puree resulted in de-esterification of the pectic substances in the cloud particles and in the serum. DE of serum pectin decreased faster and to a greater extent than that of insoluble pectin (Table 12). De-esterification of serum pectin occurred within 5 min and further incubation did not result in more saponification, whereas DE of cloud pectin decreased gradually. This indicates that PE attacks firstly the soluble pectin which is a better substrate than the insoluble pectin. Similar results were obtained by Krop (1974). The lowest DE reached by mould PE in the soluble pectin after three hours of incubation was 28.0%, indicating that PE could not saponify all the methyl esters of the pectin molecule. This may be due to the presence of neutral sugar side chains which restrict the PE from

Table 12. Changes in polysaccharide constituents and protein content of the water-insoluble fraction and DE of soluble pectin of apricot puree AIS treated with mould PE for 3 hr at 40 °C. (All weights are expressed as mg/g original AIS.)

	Incubation time (min)						
	0	5	15	30	60	120	180
Weight of residual cloud particles	695.3	698.9	706.6	687.5	677.1	689.6	685.6
AGA	169.9	163.0	167.4	158.6	163.1	159.0	164.1
Neutral sugars							
Rhamnose	15.2	14.3	16.6	15.4	14.8	15.3	14.6
Arabinose	56.1	59.1	60.1	60.9	54.0	54.2	59.1
Xylose	20.1	21.5	22.8	22.5	21.5	20.8	22.2
Mannose	19.0	17.5	20.1	19.7	17.8	19.0	17.4
Galactose	33.0	31.1	35.4	34.4	31.5	30.2	29.9
Glucose	180.0	178.8	185.2	177.7	174.0	175.5	178.2
Protein	68.1	67.3	63.9	66.3	62.8	82.7	66.4
Cellulose	216.0	---	---	---	---	---	---
DE(%)							
Insoluble pectin	63.9	50.7	50.1	48.9	43.8	39.3	37.3
Soluble pectin	76.1	28.6	31.2	30.3	29.5	29.2	28.0

saponifying the methylgalacturonides in the main chain. Ishii *et al.* (1978) also found that purified PE isolated from a culture media of *Aspergillus japonicus* reached a final DE of 38% after 6 hr of enzyme action on highly esterified pectin (DE 75%). Versteeg (1979) reported that pectins of varying degree of esterification were not fully saponified by orange PE and the residual DE was 10-15%. The results presented in Table 12 indicate that the enzyme did not affect the polysaccharide composition and the protein content of the cell wall.

In a preliminary experiment, crude orange PE was added to apricot nectar and changes in the DE of cloud particles and serum pectins monitored. The lowest DE achieved by orange PE was 48.1% and 63.9% for cloud particles and serum pectins, respectively. Thus it would seem that orange PE was less effective in de-esterifying pectin of apricot nectar than was mould PE. As stated in Section 2.1, orange PE liberates blocks of galacturonic acids which inhibit PE action (Versteeg, 1979), but this appears not to be the case with mould PE (Ishii *et al.*, 1978)

Mould PE did not affect cloud loss (Fig. 19). The clarification mechanism in orange juice which involves the formation of calcium pectate gel as a result of orange PE action is not applicable here. Further, the addition of a chelating agent (ammonium oxalate) to apricot nectar not treated with mould PE did not prevent or delay clarification. Yet Krop and Pilnik (1974a) found that addition

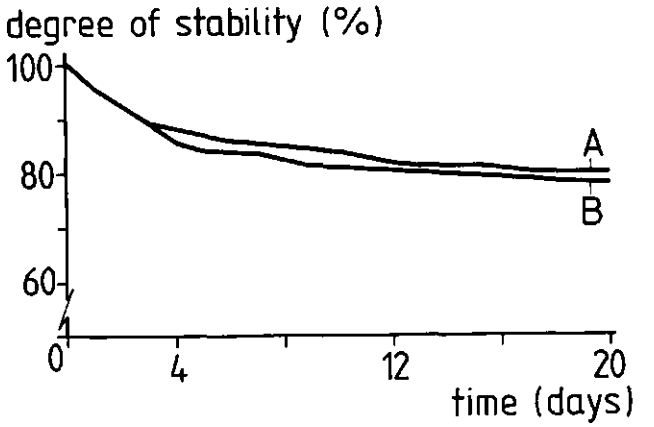


Fig. 19. Effect of PE on the cloud stability of apricot nectar: A. Untreated; B. mould PE treated.

of ammonium oxalate to orange juice prevented clarification in spite of PE activity. Therefore, it can be assumed that the clarification mechanism in apricot nectar is different to that in orange juice.

4.5.2 Effect of Rohament P

As Rohament P is an almost pure PG (Table 10) it was used in this experiment to demonstrate the effect of mould PG on polysaccharide changes.

Changes in polysaccharides and protein content of the cloud particles. When Rohament P was added to the AIS of apricot puree the weight of the residual cloud particles decreased (Table 13). The decrease in the polysaccharide constituents was mainly in AGA, rhamnose, arabinose and to a lesser extent in galactose, but the amount of xylose, mannose, and glucose remained constant. After 5 min of Rohament P action, AGA content had dropped to 25% and arabinose to 65%, and after 3 hr 7% of pectin and 28% of arabinose remained. A similar relationship was found in the decrease in rhamnose and galactose (Table 13). This suggests that the pectic polysaccharides released after 5 min of Rohament P action are relatively poor in neutral sugar side chains and are more accessible to Rohament P than those released latter which are rich in neutral sugar side chains.

Table 13. Changes in polysaccharide constituents and protein content of the water-insoluble fraction and DE of soluble pectin of apricot puree AIS treated with Rohament P for 3 hr at 40 °C (All weights are expressed as mg/g original AIS.)

	Incubation time (min)						
	0	5	15	30	60	120	180
Weight of residual cloud particles	695.3	517.1	499.3	481.6	449.1	428.4	415.1
AGA	169.9	43.2	31.0	30.1	17.6	14.9	12.5
Neutral sugars							
Rhamnose	15.2	10.3	9.9	8.6	8.5	7.6	6.7
Arabinose	56.1	36.6	37.3	23.6	19.9	18.6	15.8
Xylose	20.1	18.8	21.7	22.2	20.5	20.3	21.0
Mannose	19.0	17.4	18.5	19.8	19.3	18.3	16.9
Galactose	33.0	27.4	28.7	27.9	24.7	23.8	23.0
Glucose	180.0	171.6	187.2	183.1	181.9	183.6	176.3
Protein	68.1	70.4	68.5	67.9	65.6	63.1	63.0
Cellulose	216.0	222.7	---	214.6	---	212.4	206.6
DE (%)							
Insoluble pectin	63.9	48.0	---	---	40.2	---	32.6
Soluble pectin	76.1	73.9	70.3	67.0	66.8	66.7	67.3

Cellulose and insoluble protein decreased slightly as a result of Rohament P action (Table 13), mainly because of the cellulase and protease activities present in this preparation.

As a result of solubilization of pectic polysaccharides from the cloud particles pectins with progressively decreasing DE were left in the residual particles (Table 13). It was expected that this pectin would have been more susceptible to Rohament P action but it may well be protected from solubilization by Rohament P by protopectin binding or by the presence of hairy regions. Strübi (1976) found also that under Irgazyme M-10 action, pectin having a high DE was released from apple cell walls but pectin having lower DE remained in the residual cell wall material.

Changes in serum pectin. Gel permeation chromatography on Sephacryl S-200 of serum pectin of untreated apricot puree AIS showed that the soluble pectin was eluted as one peak in the void volume (Fig. 20). Although Rohament P solubilized a considerable amount of pectic polysaccharides from the cloud particles to the serum, all the soluble fragments voided the column (Fig. 21). The elution pattern of the serum pectin did not change very much after 3 hr of enzyme action, because Rohament P has mainly PG activity and only

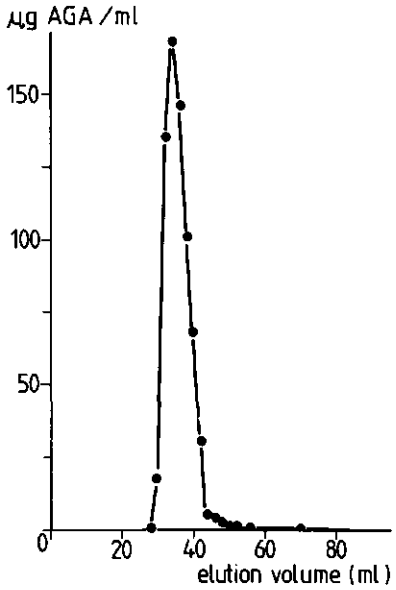


Fig. 20. Elution pattern of serum pectin of apricot nectar on Sephacryl S-200.

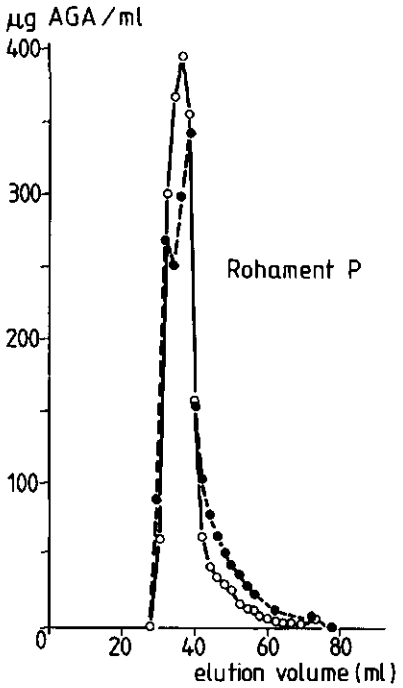


Fig. 21. Elution pattern of serum pectin of apricot nectar treated with Rohament P for 5 min (o—o) and 3 hr (●—●).

trace activities of PE and PL. The DE of serum pectin after 3 hr of Rohament P action was 67.3% (see Table 13) which is too high to be extensively depolymerized by the PG activity present in this preparation.

Effect on cloud stability. The addition of Rohament P to apricot nectar improved the level of cloud stability but did not stabilize the nectar completely (Fig. 22). This confirms the results of the experiment described in Section 4.3, Fig. 13. The sedimented cloud particles did not form a gel. The cloud particles of both untreated nectar and Rohament P treated nectar were examined under the microscope. The cloud particles of untreated nectar were composed of cell aggregates and also some individual cells characterized by a thick cell wall (Fig. 23a), and those of nectar treated with Rohament P were composed mainly of individual cells characterized by thin cell walls (Fig. 23b).

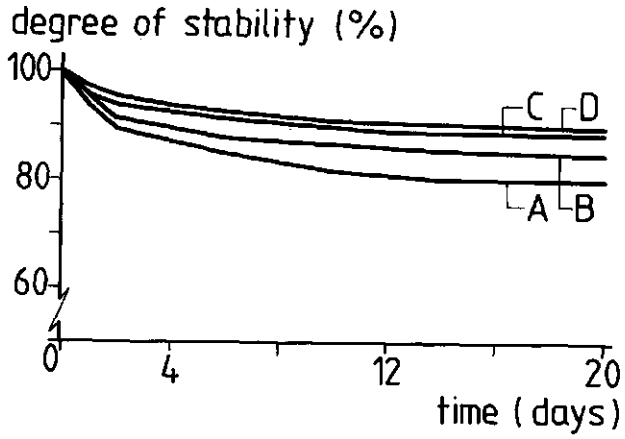
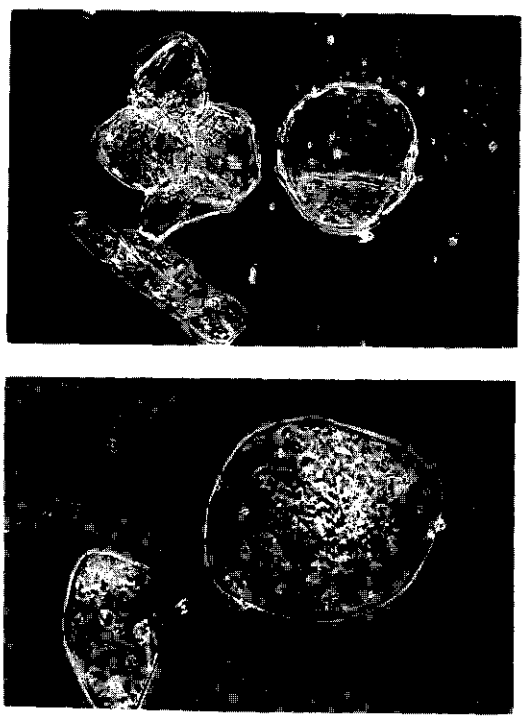


Fig. 22. Effect of Rohament P on cloud stability of apricot nectar: A. Untreated; B, C, and D. treated with Rohament P for 1, 2 and 3 hr respectively.



a

b

Fig. 23. Cloud particles of apricot nectar: a. Untreated; b. treated with Rohament P.

The cell walls were intact. The high proportion of individual cells is due to the cell-separating effect of Rohament P, as Rohament P acts on and solubilizes, especially middle lamella pectin.

4.5.3 Effect of purified PL

Changes in polysaccharides and protein content of the cloud particles. Changes in residual weight of the cloud particles, AGA, neutral sugars, protein and cellulose content of the cloud particle AIS and also changes in DE of insoluble and soluble pectin as a result of PL action are presented in Table 14. After 5 min, rhamnose, arabinose and AGA were released, but galactose was released only after 15 min of enzyme action. The relative proportion of rhamnose and arabinose released with AGA after 3 hr was higher than that released after 5 min. These results indicate that the pectin released first was rhamnogalacturonan with a small amount of neutral sugar side chains not including galactose. This suggests that in apricot fruit there are pectic substances which contain only arabinan side chains.

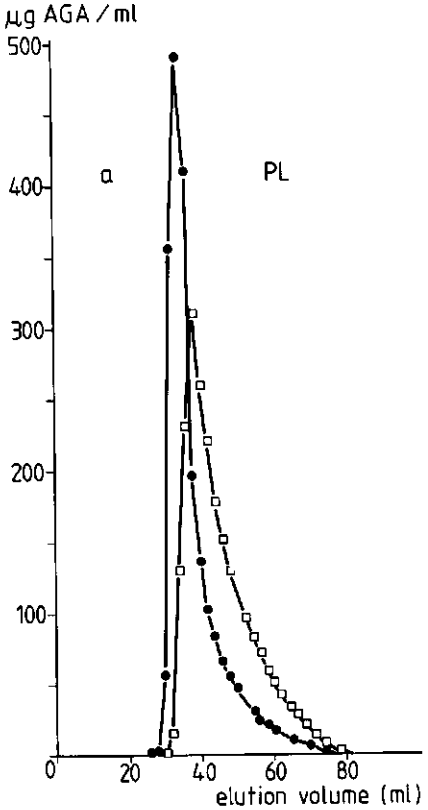
Table 14. Changes in polysaccharide constituents and protein content of the water-insoluble fraction and DE of soluble pectin of apricot puree AIS treated with PL for 3 hr at 40 °C (All weights are expressed as mg/g original AIS.)

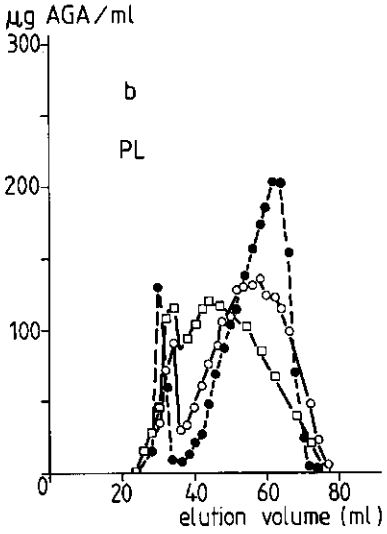
	Incubation time (min)						
	0	5	15	30	60	120	180
Weight of residual cloud particles	695.3	574.3	498.2	452.8	434.4	423.3	424.4
AGA	169.9	73.1	36.8	22.9	16.5	13.1	12.9
Neutral sugars							
Rhamnose	15.2	12.5	10.3	9.1	6.9	7.6	6.8
Arabinose	56.1	51.9	38.1	23.8	20.2	16.6	12.9
Xylose	20.1	24.4	25.4	23.6	25.8	23.8	22.3
Mannose	18.9	18.8	19.3	19.0	18.6	20.4	19.6
Galactose	33.0	33.1	29.7	26.7	25.4	25.4	22.1
Glucose	180.0	189.1	189.0	181.6	184.2	184.9	179.3
Protein	68.1	70.9	68.6	68.2	67.5	61.9	67.2
Cellulose	215.9	208.3	---	203.8	204.7	206.3	205.2
DE(%)							
Insoluble pectin	63.9	54.6	37.2	29.3	24.5	13.2	6.3
Soluble pectin	76.1	71.1	69.9	64.0	64.8	69.8	62.6

The amount of xylose and mannose increased slightly with time, but the amount of glucose remained the same. Probably because of the change in the structure of the insoluble solids as a result of the solubilization of the pectic polysaccharides, the remaining sugars (xylose and mannose) became more accessible. This improved formation of alditol acetate derivatives, thus explaining why the recovery of xylose and mannose was higher. Wallner & Bloom (1977) ascribed the higher proportion of xylose obtained after treating tomato cell walls with tomato PG to the disproportionate removal of galacturonic acid. Similarly, Kono & Yamazaki (1982) attributed an apparent increase in the amount of mannose and glucose in carrot cell walls as a result of pectate lyase action to the massive alteration of the plant cell wall structure by endo-pectolytic enzyme action.

The amount of cellulose decreased only slightly, but insoluble protein did not change as a result of PL action (Table 14). DE of the insoluble pectin decreased sharply after 3 hr of PL action because PL attacks pectin with high DE, thus leaving pectin with low DE in the cloud particles. The pronounced decrease in DE of serum pectin as a result of the solubilization of pectic polysaccharides from the cloud particles may be explained by the higher DE of the serum pectin (76.1%) than that of the insoluble pectin (63.9%).

Changes in serum pectin. Elution patterns after gel permeation chromatography of serum pectin degraded by PL action are shown in Fig. 24. After 5 min and 15 min of PL action the solubilized pectic polysaccharides eluted in a similar pattern to that of untreated serum pectin (Fig. 20). Further incubation resulted in massive degradation of soluble pectin (Fig. 24b), resulting in two separate fractions. One fraction was eluted in the void volume and the other was partially retained on the column. The pectin fraction which was eluted in the void volume was rich in neutral sugar side chains. The proportion of AGA in this fraction was 20.8%, 12.9%, and 11.0% of the total AGA present in the serum after 1, 2, and 3 hr respectively of PL action. To characterize this fraction, the neutral sugars which were eluted with the pectin in the void volume were determined by gas chromatography after hydrolysis as the alditol acetates. This fraction was rich in neutral sugar side chains, especially arabinose (Fig. 24c). It contained 3.2 moles of neutral sugars/mole AGA, which was predominantly arabinose, 2.4 moles arabinose/mole AGA.





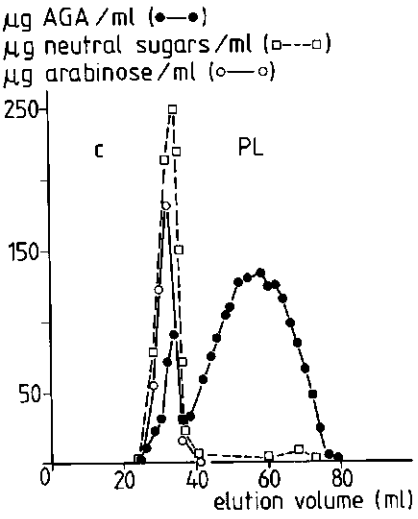


Fig. 24. Elution pattern of serum pectin of apricot nectar treated with PL: a. for 5 (•—•) and 15 min (□—□); b. for 1 (□—□), 2 (○—○) and 3 hr (•---•); c. for 2 hr. Fractions were monitored for AGA, arabinose and neutral sugars as sum of rhamnose, arabinose, xylose, mannose, galactose and glucose.

Effect on cloud stability. Addition of purified PL to apricot nectar stabilized the cloud completely during the experimental period (Fig. 25). This is in agreement with the results presented in Section 4.3. Examination under the microscope showed that the cloud particles were composed mainly of individual cells and the cell wall had been ruptured to such an extent that the intercellular components had been liberated (Fig. 26). This indicates that highly esterified pectin which can be broken down and solubilized by PL is an essential structural element of the cell walls.

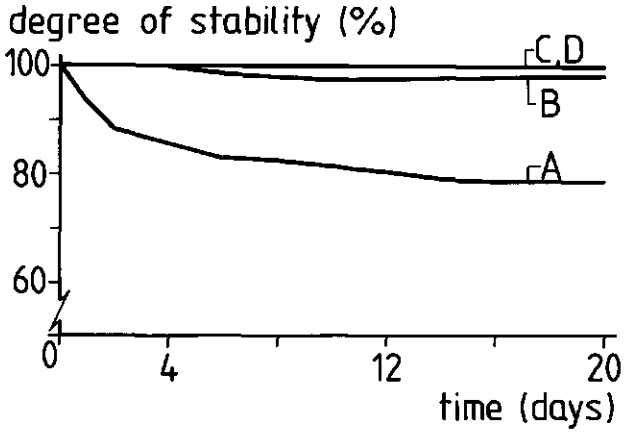


Fig. 25. Effect of PL on the cloud stability of apricot nectar: A. Untreated; B, C, and D treated with PL for 1 hr, 2 hr, and 3 hr respectively.

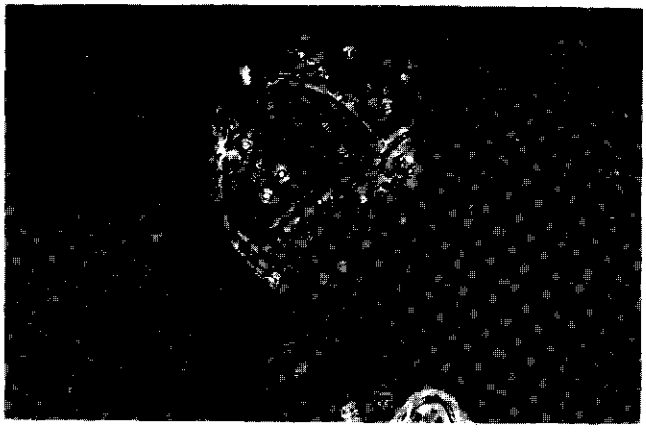


Fig. 26. Cloud particles of apricot nectar after treatment with PL.

4.5.4 Effect of Pectinex

Changes in polysaccharides and protein content of the cloud particles. Changes in cell wall constituents as a result of Pectinex action are presented in Table 15. Reduction in the residual amount of cloud particles was higher after Pectinex than after Rohament P or PL action. Treatment with Pectinex achieved the same effect within 5 min as that achieved with Rohament P or PL within 3 hr, mainly because of the high activities of PG, PE, and PL in the addition to cellulase, hemicellulase, and protease activities. The lower level of neutral sugars in the cloud particles after 3 hr of Pectinex action suggests that other polysaccharide degrading enzymes, (such as, arabinases, galactanases, and other hemicellulases) are involved in lowering the amounts of neutral sugars. However, Voragen *et al.* (1982) found that the combination of galactanase, arabinase, PE, and PG did not solubilize more polysaccharide fragments from apple cell walls than did the combination of PG and PE. The amount of cellulose and insoluble proteins also decreased (Table 15), because of cellulase and protease activities which are normally present in such commercial preparations.

Table 15. Changes in polysaccharide constituents and protein content of the water-insoluble fraction and DE of soluble pectin of apricot puree AIS treated with Pectinex for 3 hr at 40 °C (All weights are expressed as mg/g original AIS.)

	Incubation time (min)						
	0	5	15	30	60	120	180
Weight of residual cloud particles	695.3	426.5	412.1	403.5	395.9	378.5	370.7
AGA	169.9	10.5	10.3	10.2	10.9	10.2	9.9
Neutral sugars							
Rhamnose	15.2	7.0	7.7	7.3	7.6	5.9	5.8
Arabinose	56.1	15.2	14.1	11.9	10.5	7.7	6.5
Xylose	20.1	20.6	25.3	24.0	22.2	22.3	19.6
Mannose	19.0	17.2	18.3	19.7	18.1	17.4	18.0
Galactose	33.0	22.9	23.5	25.7	21.6	21.6	20.0
Glucose	180.0	173.3	184.9	203.7	173.4	173.0	169.1
Protein	68.1	69.1	68.8	65.0	64.9	59.1	56.8
Cellulose	216.0	---	---	208.8	195.9	184.0	182.2
DE (%)							
Insoluble pectin	63.9	0.0	0.0	0.0	0.0	0.0	0.0
Soluble pectin	76.1	24.1	22.0	22.8	20.1	16.5	15.8

The DE of soluble pectin decreased rapidly from 76.1% to 24.1% after 5 min of Pectinex action and subsequently decreased gradually reaching 15.8% after 3 hr. The DE of soluble pectin of Pectinex-treated sample was lower (Table 15) than that treated with mould PE alone (Table 12).

Changes in serum pectin. The elution pattern after gel permeation chromatography of serum pectin of apricot puree AIS treated with Pectinex after incubation for 0.5, 1 and 2 hr is shown in Fig. 27a. The pectin fraction which was eluted in

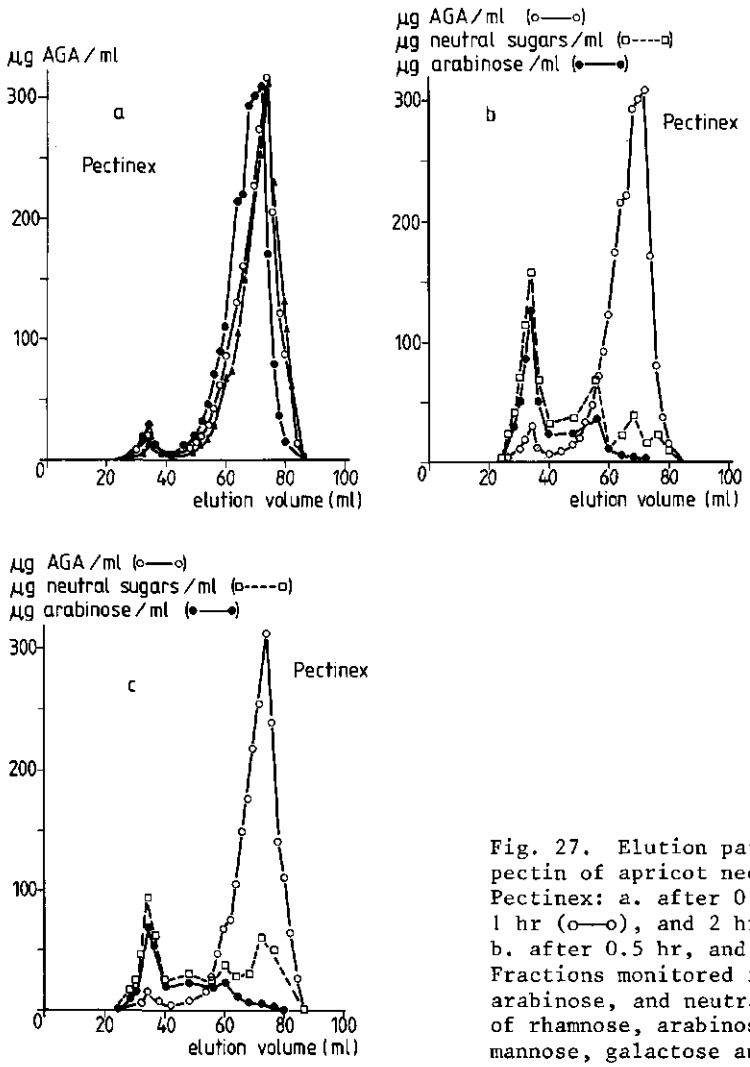


Fig. 27. Elution pattern of serum pectin of apricot nectar treated with Pectinex: a. after 0.5 hr (•—•), 1 hr (o—o), and 2 hr (▲—▲); b. after 0.5 hr, and c. after 2 hr. Fractions monitored for AGA, arabinose, and neutral sugars as sum of rhamnose, arabinose, xylose, mannose, galactose and glucose.

the void volume was small compared with the corresponding fraction of PL-degraded serum (Fig. 24b) mainly because of the presence of various activities of polysaccharide-degrading enzymes in the Pectinex preparation. This fraction consisted of 3.3%, 2.1% and 1.6% of the total AGA in serum pectin after 0.5, 1, and 2 hr respectively, of Pectinex action. The amount of neutral sugars of the fractions collected from gel-filtration on Sephacryl S-200 after 0.5 and 2 hr of Pectinex treatment were analysed by gas chromatography. The distribution of AGA, arabinose, and total neutral sugars after Pectinex treatment are presented in Fig. 27b and c. The pectin fraction eluted in the void volume was rich in neutral sugars, especially arabinose. After 0.5 hr this fraction contained 7.0 moles neutral sugars/mole AGA (mostly arabinose 5.5 moles/mole AGA), and after 2 hr 7.6 moles neutral sugars/mole AGA (of which 6.1 moles arabinose/mole AGA).

Effect on cloud stability. Pectinex activity completely stabilized the cloud of apricot nectar (Fig. 28). This is in agreement with the results presented in Section 4.3 (Fig. 13). Under microscope examination, the cell wall of the cloud particles was shown to have been ruptured (Fig. 29).

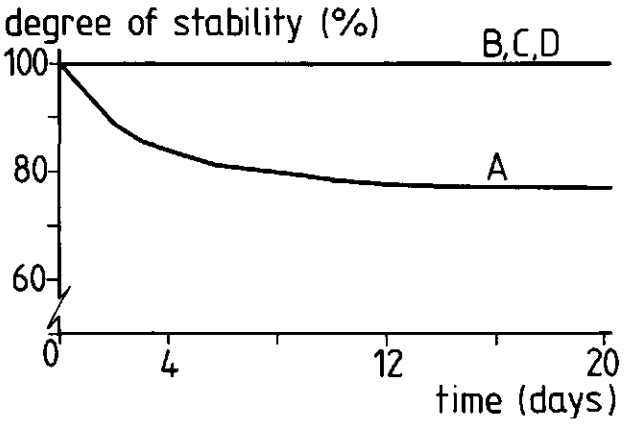


Fig.28. Effect of Pectinex on the cloud stability of apricot nectar: A. Untreated; B, C, and D treated with Pectinex for 1 hr, 2 hr, and 3 hr respectively

Fig. 29. Cloud particles after treatment with Pectinex.

4.5.5 Effect of protease

Changes in polysaccharides and protein content of the cloud particles. Addition of protease to AIS of apricot puree led to a strong decrease in cell wall proteins (Table 16). Since 10.2% of the AGA was shown to have been released from the cloud particles after 3 hr of enzyme action, the secondary activities of pectolytic enzymes in the protease preparation were assayed. No PE or PL was detected, but 0.44 units of PG per gram protease was found. The amount of protease added to the AIS was 40 mg which would preclude PG activity from being responsible for the pectin solubilization. Probably, solubilization of cell wall protein rendered part of the cell wall pectin soluble in the serum. Treatment of cell wall with proteolytic enzymes has been found to reduce its tensile strength (Preston, 1974) and to liberate pectic and hemicellulose fragment with peptides (Keegstra *et al.*, 1973). In the present study, the amount of arabinose increased as a result of protease action, and the amounts of other neutral sugars remained the same. This may be attributed to degradation of some peptide chains linked to cell wall polysaccharides (arabinose-hydroxyproline linkages) as shown in the model of Fig. 3. Removal of these peptides improved the recovery of arabinose in sugar analysis.

Table 16. Changes in polysaccharide constituents and protein content of the water-insoluble fraction and DE of soluble pectin of apricot puree AIS treated with protease for 3 hr at 40 °C (All weights are expressed as mg/g original AIS.)

	Incubation time (min)						
	0	5	15	30	60	120	180
Weight of residual cloud particles	695.3	680.4	673.3	663.7	651.0	636.8	630.6
AGA	169.9	162.1	170.9	168.5	170.4	162.2	152.6
Neutral Sugars							
Rhamnose	15.2	14.1	15.8	13.9	14.8	15.3	14.8
Arabinose	56.1	62.1	74.9	54.7	67.8	63.7	63.8
Xylose	20.1	22.3	26.2	24.6	24.7	20.5	24.7
Mannose	19.0	19.3	20.3	19.4	22.3	19.6	18.3
Galactose	33.0	32.5	35.5	34.4	36.9	33.7	32.0
Glucose	180.0	178.0	196.8	185.0	179.1	173.4	179.5
Protein	68.1	65.3	60.3	53.5	64.9	40.5	39.8
Cellulose	216.0	---	---	---	---	---	---
DE(%)							
Insoluble pectin	63.9	62.6	62.2	63.0	61.7	63.0	63.5
Soluble pectin	76.1	72.4	73.3	74.0	76.0	74.5	73.4

Selvendran (1975) reported that cell wall protein interfered with methylation of neutral sugars and that complete removal of cell wall protein was necessary for a high recovery of the sugars.

Changes in serum pectin. Protease treatment had very little effect on the serum pectin. The elution pattern of the serum pectin was similar to that of untreated serum pectin (Fig. 20).

Effect on cloud stability. Protease treatment also had very little effect on the cloud loss of apricot nectar (Fig. 30). The sedimentation of cloud particles and gel formation were similar in protease-treated and untreated nectar.

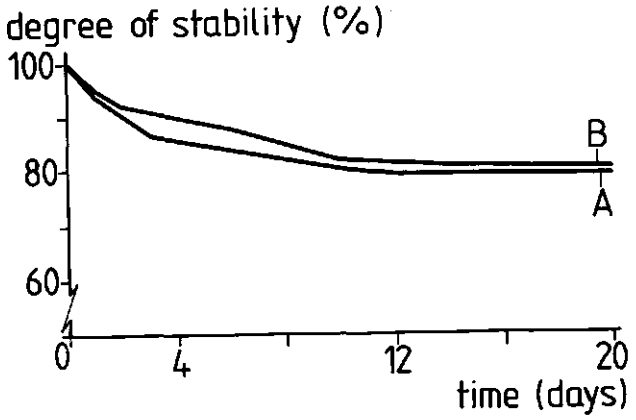


Fig. 30. Effect of protease on the cloud stability of apricot nectar: A. Untreated; B. treated with protease for 3 hr.

4.6 ROLE OF ARABINAN SIDE CHAINS ON CLOUD STABILITY OF APRICOT NECTAR

Commercial and purified pectolytic enzyme preparations have been demonstrated to solubilize considerable amounts of pectin with related neutral sugars from the cloud particles. Mainly arabinose has been shown to be released by these enzyme preparations. Pectinex which was found to stabilize cloud particles released more arabinose than Rohament P which was found not to stabilize cloud particles. Therefore, purified exo-arabinase (1.7 units/100 g puree) was added alone and in combination with purified PG (25 units/100 g puree) to elucidate the rôle of arabinan side chains in gel formation and in cloud stability of apricot nectar.

4.6.1 Effect of purified exo-arabinase

Exo-arabinase isolated from Pectinase no. 29 (Gist-Brocades) was added to apricot puree as described in Section 3.2.1. After 1, 2, and 3 hr samples were withdrawn and nectar was prepared as described in Section 3.2.1 for nectar III (Fig. 7). The changes in carbohydrate composition of the cloud particles were studied. Only arabinose was solubilized from the cloud particles as a result of exo-arabinase action, while the amount of other neutral sugars, for example, rhamnose, xylose, galactose and glucose, remained constant (Table 17). The amount of AGA in cloud particles and the DE of the pectin did not alter even after 3 hr of enzyme action.

Table 17. Changes in the amount of AGA and neutral sugars of cloud particle AIS of apricot nectar made from apricot puree treated with exo-arabinase for 3 hr at 40 °C. (All weights are expressed as mg/100 ml nectar.)

	Incubation time (min)			
	0	60	120	180
AGA	163.7	144.2	141.3	147.6
Neutral sugars				
Rhamnose	18.5	18.3	16.8	18.5
Arabinose	56.2	31.7	27.9	27.3
Xylose	28.8	28.4	27.4	28.3
Mannose	19.4	19.0	18.4	20.0
Galactose	40.7	41.9	40.5	41.4
Glucose	216.6	227.7	218.5	230.9
DE(%)	63.1	61.2	62.1	62.2

Exo-arabinase did not stabilize the cloud particles (Fig. 31) and did not inhibit gel formation. When examined under the microscope, cloud particles in exo-arabinase treated and untreated nectar were shown to be similar (Fig. 23a). The mode of action of exo-arabinase was studied using two substrates. One substrate was haze-arabinan which is formed in apple juice. This has been isolated and characterized by Voragen *et al.* (1982) and found to be linear arabinan with α-1,5-linkages. The other substrate was the ultrafiltration retentate of apple juice derived from apple pulp treated with pectolytic and

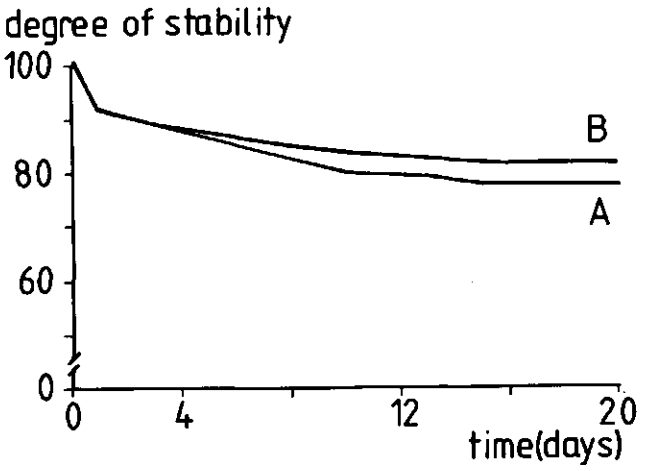


Fig. 31. Effect of exo-arabinase on the cloud stability of apricot nectar: A. Untreated; B. treated with exo-arabinase for 3 hr.

Table 18. Sugar and glycosidic linkage composition of ultrafiltration retentate (Voragen *et al.*, 1985)

Sugar composition (as mole %)		Glycosidic linkage composition (as mole %)	
Rhamnose	5.6	1,2,4-Rha*p*	100
Arabinose	55.1	T-Ara*f*	25
		1,2-Ara*f*	1
		1,3-Ara*f*	8
		1,5-Ara*f*	47
		1,3,5-Ara*f*	11
		1,2,5-Ara*f*	3
		1,2,3,5-Ara*f*	5
Xylose	8.4	T-Xyl*p*	65
		1,4-Xyl*p*	32
		1,2-Xyl*p*	3
Galactose	9.9	T-Gal*p*	45
		1,3-Gal*p*	19
		1,4-Gal*p*	14
		1,6-Gal*p*	4
		1,2,4-Gal*p*	4
		1,3,6-Gal*p*	14
Galacturonic acid	20.9	1,4-Gal*p*A*	80
		1,3,4-Gal*p*A*	20

* Determined after carboxyl reduction.

cellulolytic enzymes. The sugar and glycosidic linkage composition of ultrafiltration retentate is given in Table 18 (Voragen *et al.*, 1985). After incubation for 72 hr with exo-arabinase, the haze-arabinan was completely degraded into arabinose. Similarly the retentate arabinan was degraded to 80%. This indicates that the exo-arabinase isolated from Pectinase no. 29 splits 1,5- and 1,3-linkages and can therefore degrade both linear and branched arabinan.

4.6.2 Effect of purified PG + purified exo-arabinase

The combined action of purified PG and purified exo-arabinase released higher amounts of rhamnose, arabinose, and AGA than did purified PG only (Tables 19 and 20). Also, DE of insoluble pectin decreased more under combined PG and exo-arabinase action than under PG action only. Exo-arabinase has been demonstrated to release only arabinose (see Section 4.6.1), and therefore there

Table 19. Changes in the amount of AGA and neutral sugars of cloud particle AIS of apricot nectar made from apricot puree treated with PG for 3 hr at 40 °C. (All weights are expressed in mg/100 ml nectar.)

	Incubation time (min)			
	0	60	120	180
AGA	161.6	51.5	44.4	41.4
Neutral sugars				
Rhamnose	21.0	13.1	14.4	13.1
Arabinose	58.6	33.5	31.8	29.3
Xylose	34.7	35.9	35.4	36.4
Mannose	23.6	24.3	24.9	23.5
Galactose	43.8	37.1	36.1	37.3
Glucose	240.8	230.6	243.9	240.8
DE(%)	63.0	44.4	41.6	40.1

Table 20. Changes in the amount of AGA and neutral sugars of cloud particle AIS of apricot nectar made from apricot puree treated with purified PG + exo-arabinase for 3 hr 40 °C (All weights are expressed as mg/100 ml nectar.)

	Incubation time (min)			
	0	60	120	180
AGA	161.6	42.5	35.4	34.0
Neutral sugars				
Rhamnose	21.0	11.9	10.9	9.7
Arabinose	58.6	19.4	14.5	13.5
Xylose	34.7	30.5	31.1	28.4
Mannose	23.6	22.3	21.5	19.9
Galactose	43.8	34.9	34.8	33.4
Glucose	240.8	240.7	243.5	237.6
DE(%)	63.0	40.4	37.9	37.1

would seem to be a synergistic effect of PG and exo-arabinase on pectin solubilization. The combined action of PG and exo-arabinase completely stabilized the cloud particles (Fig. 32), and while PG alone improved cloud stability, it did not stabilize it completely. The cloud particles of apricot nectar treated with PG + exo-arabinase were shown to be composed mainly of individual cells and the cell walls were shown to have been partly ruptured (Fig. 33).

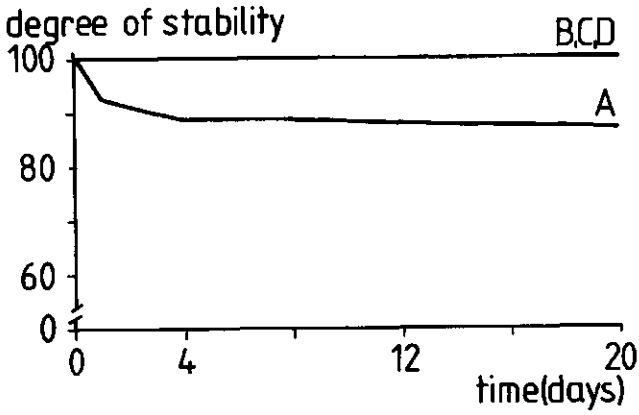


Fig. 32. Effect of PG + exo-arabinase on the cloud stability of apricot nectar: A. Untreated; B, C, and D treated with PG + exo-arabinase for 1, 2, and 3 hr respectively.

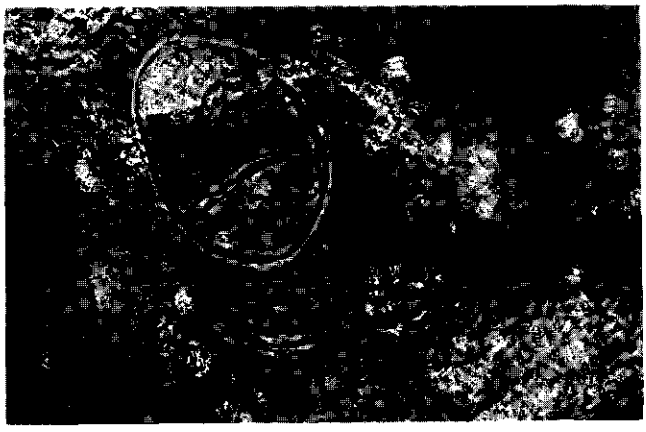


Fig. 33. Cloud particles of apricot nectar after treatment with PG + exo-arabinase.

4.7 EFFECT OF STABILIZING AND NON-STABILIZING ENZYMES ON THE CHEMICAL COMPOSITION OF SERUM AND OF CLOUD PARTICLES

In this section the enzyme systems examined in Section 4.5 for their effect on AIS of apricot puree were added directly to apricot puree to compare their effect on the puree itself. Purified mould PG was also used. The various enzyme systems were added to the puree and each mixture incubated for 2 hr at 40 °C. Subsequently the nectar was prepared as described in Section 3.2.1 (Nectar III, Fig. 7) and then separated into serum and cloud particles. AIS was prepared from both fractions and subjected to further examination. Purified pectolytic enzymes (PE, PG, and PL) were added in amounts to give the same level of activity as present in 0.1 ml Pectinex (Table 10). The amount of exo-arabinase was 2 units/100 g puree.

4.7.1 Changes in the cloud particles

Changes in the amount of residual cloud particles, neutral sugars and also the DE of the residual insoluble pectin are set out in Table 21. The amount of arabinose decreased as a result of exo-arabinase action while the amount of other neutral sugars and AGA remained constant. All pectolytic enzymes used in this experiment solubilized cloud particles to a varying extent. The solubilized components were mainly constituents of pectic polysaccharides, rhamnose, arabinose, and AGA being solubilized most, and galactose the least (Fig. 34). PG was less active in solubilizing these components than PL, PG + exo-arabinase, PG + mould PE, or Pectinex. The combined action of orange PE and PG had little effect on either solubilization or on DE of the residual pectin, whereas the combination with mould PE did.

DE of the insoluble pectin decreased correspondingly with the release of polysaccharides, and was lower in nectar treated with PG + exo-arabinase, PG + mould PE, PL, and Pectinex than with PG. From these data, it can be derived that the removal of arabinose side chains permits PG to release not only non-esterified galacturonides but also fragments which are esterified to some extent.

Table 21. Changes in the residual weight, and amount of AGA and neutral sugars of cloud particles AIS of apricot nectar made from apricot puree treated with various enzyme systems for 2 hr at 40 °C (All weights are expressed as mg/300 ml nectar.)

Enzyme treatment	Residual cloud particle AIS	AGA	Neutral sugars						DE(%)
			Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	
Untreated	1307	277.1	50.6	111.0	52.1	45.5	71.1	393.0	63.2
Exo-arabinase	1237	266.0	49.3	54.9	54.6	45.1	72.6	403.0	61.8
Purified PG	912	52.7	27.6	47.3	51.2	44.2	55.6	391.2	45.3
PG + orange PE	914	52.1	28.3	48.4	52.5	46.1	53.4	383.7	43.6
PG + exo-arabinase	845	39.2	21.1	21.0	48.3	43.4	46.9	376.1	36.7
PG + mould PE	867	26.4	20.4	28.5	48.4	42.8	49.2	369.1	20.3
PL	872	31.1	19.9	33.2	48.8	44.7	50.6	390.5	20.6
Pectinex	811	22.1	15.7	20.3	44.1	40.6	45.0	370.0	19.7

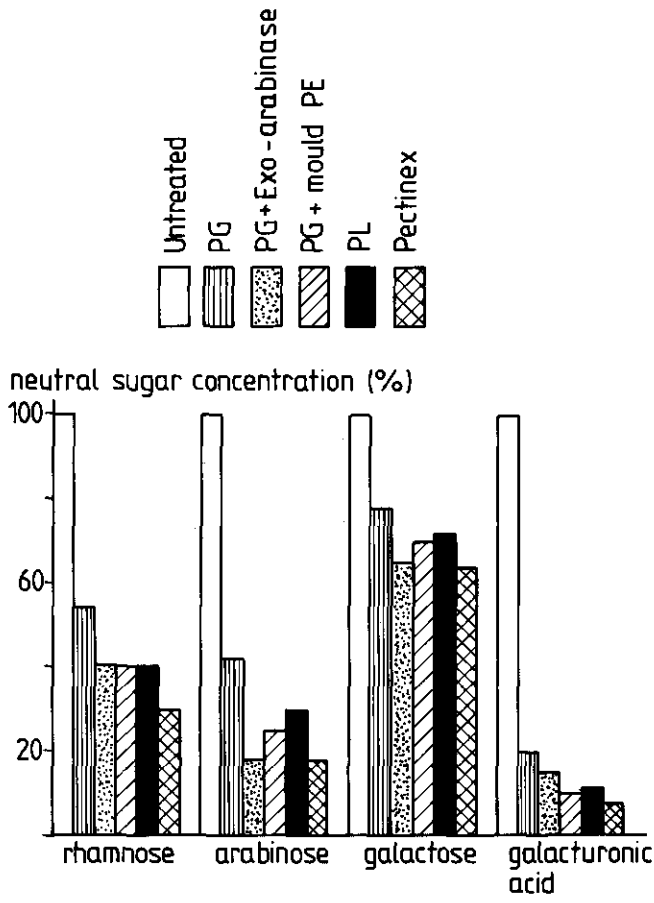


Fig. 34. Changes in the amounts of neutral sugars in the cloud particles of apricot nectar after treatment with different enzyme systems for 2 hr at 40 °C (The amount present in the untreated is considered to be 100%.)

4.7.2 Changes in serum polysaccharides

Various enzyme systems have a two-stage action: solubilization of polysaccharides from the cell wall; and degradation of the solubilized material into smaller fragments. These fragments contain a fraction of low molecular weight, which is soluble in 80% ethanol, and a fraction of high molecular weight, which is precipitable in 80% ethanol. The high molecular weight fraction is the serum AIS which was analysed in this experiment.

The weight of serum AIS and its content of AGA in the samples treated with

PG + exo-arabinase, PG + mould PE, PL, and Pectinex were both lower than in that treated with PG (Table 22), because of the extensive degradation of the pectin molecule to small fragments rendering it not precipitable in 80% ethanol. Serum of nectar subjected to the activity of PG + mould PE, PL, and Pectinex gave a sandy precipitate, while that subjected to exo-arabinase, PG, and PG + exo-arabinase was gel-like. Thus, it may be concluded that PG acted as a macerating enzyme and solubilized pectic substances from the cell wall without degrading it extensively into smaller fragments. On the other hand, PG + mould PE, PL, and Pectinex not only solubilized the pectic polysaccharides but also degraded them to smaller fragments soluble in 80% ethanol. These findings were confirmed by gel permeation chromatography of the solubilized material from the cloud particles as described below. They are also in agreement with the proposition put forward by Voragen & Pilnik (1981) and Dongowski & Bock (1981) that PG is a macerating enzyme, and PE + PG, and PL are used for pulp treatment to increase juice yield (Section 2.5.2).

The amount of galacturonides and serum AIS were both lower after PL treatment than after PG + mould PE, even though almost the same amount of material was solubilized from the cloud particles (Table 21 and Table 22). It would seem that PL can act on highly esterified pectin regions in which neutral sugar side chains are concentrated (hairy regions). The high amounts of neutral sugars in the alcohol precipitable serum fraction (Table 23) indicate that the highly esterified fragments between branch points which were depolymerized by PL were homogalacturonan.

Table 22. Changes in weight of serum AIS, its content of AGA and DE of AIS pectin of apricot nectar made from apricot puree treated with various enzyme systems for 2 hr at 40 °C (All weights are expressed as mg/300 ml nectar.)

Enzyme treatment	Weight of serum AIS	AGA	DE(%)
Untreated	211	125.7	69.6
Exo-arabinase	195	122.7	70.0
Purified PG	590	317.5	68.8
PG + exo-arabinase	514	280.9	65.7
PG + mould PE	338	165.4	46.3
PL	233	101.9	44.0
Pectinex	92	37.9	24.5

Table 23. Changes in the amounts of neutral sugars in serum AIS of apricot nectar made from apricot puree treated with various enzyme systems after incubation for 2 hr at 40 °C (All weights are expressed as mg/300 ml nectar.)

Enzyme treatment	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
Untreated	2.2	5.4	1.5	0.4	4.7	3.1
Exo-arabinase	3.0	3.4	1.2	0.3	5.1	2.1
Purified PG	12.1	54.0	4.4	0.7	17.6	6.2
PG + exo-arabinase	12.3	20.3	5.7	0.3	17.5	5.7
PG + mould PE	10.6	43.0	4.4	0.7	14.1	5.7
PL	12.0	46.1	3.1	0.5	13.3	2.2
Pectinex	3.8	15.6	2.0	0.1	5.1	0.8

The amount of serum AIS obtained after Pectinex treatment was only 15.9% of the amount of serum AIS obtained after PG treatment (Table 22).

The fact that Pectinex degraded both the insoluble and the soluble pectin almost completely, and PL, and PG + mould PE only partially degraded the pectin reflects the importance of the wide spectrum pectolytic activity and the hemicellulases present in Pectinex. These enzymes act on the side chains, for example, arabinan side chains, and liberate the main galacturonan backbone which is degraded further by various pectolytic enzymes (PE, PG and PL). Gross & Wallner (1979) reported that galactan or arabinogalactan may regulate PG activity by restricting the access of the enzyme to the substrate within the complex of wall polysaccharides. Also, arabinan side chains could provide protection against PG action (Ahmed & Labavitch, 1980).

The amounts of the neutral sugars in serum AIS after treatment with various enzyme systems are presented in Table 23. The amount of arabinose decreased as a result of exo-arabinase treatment. The amount of neutral sugars in serum of untreated nectar was much lower than that treated with various pectolytic enzymes. This suggests that the enzymatically released pectin is richer in neutral sugar side chains than the water soluble pectin of untreated nectar. The amount of AGA of serum AIS increased 2.5 times in PG-treated nectar compared to untreated, and the amount of rhamnose, arabinose and galactose increased 5, 10 and 3.7 times respectively.

The amount of arabinose in serum AIS was lower in nectar treated with PG + exo-arabinase, PG + mould PE, PL, and Pectinex than in PG-treated nectar. The main reason is that small fragments (short galacturonan backbone with neutral sugar side chains) were further degraded to alcohol soluble fragments.

The results presented in Tables 21, 22 and 23 are summarized in Table 24 and show the changes produced by degradation of pectin fractions to alcohol soluble

Table 24. Total amount of AIS with AGA and neutral sugars content of serum and cloud particles recovered from 300 ml nectar prepared from apricot puree treated with various enzyme systems for 2 hr at 40°C (All weights are expressed as mg/300 ml nectar.)

Enzyme treatment	Weight of total AIS	AGA	Neutral sugars					
			Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
Untreated	1518	402.8	52.8	116.3	53.6	45.9	75.8	396.1
Exo-arabinase	1492	388.2	52.3	58.3	54.8	45.7	77.8	405.1
Purified PG	1485	370.2	39.8	101.3	55.5	44.9	73.2	397.2
PG + exo-arabinase	1359	320.1	33.5	41.3	54.0	43.6	64.4	381.8
PG + mould PE	1205	191.9	30.9	71.9	52.8	43.5	63.3	374.9
PL	1105	133.0	31.8	79.3	51.8	45.2	63.9	392.7
Pectinex	903	59.9	19.4	35.9	46.2	40.6	50.1	370.8

fragments. Pectin is again shown to be degraded only when methoxyl groups and/or arabinan side chains are removed. Exo-arabinase alone released arabinose from side chains. A high amount of arabinose was left in those fractions where particularly the smooth homogalacturonan part of the molecule has been degraded. The small changes in the other neutral sugars indicate that arabinose is the main constituent of the side chains.

Results of gel permeation chromatography of serum pectin on Sephacryl S-200. Serum AIS isolated from apricot nectar after treatment with various enzyme systems was subjected to gel permeation chromatography to study the extent of degradation. The polysaccharides in untreated serum were eluted as a single peak in the void volume as shown in Fig. 35a. This pattern did not change when exo-arabinase was added to the nectar (Fig. 35b). However, PG caused limited degradation in serum pectin and a sharp peak in the void volume rich in neutral sugars occurred (Fig. 35c). The elution pattern of nectar treated with PG + exo-arabinase was similar to that of nectar treated with PG (Fig. 35d). Fragments which had been attacked by PG + exo-arabinase but not by PG alone were soluble in 80% ethanol and were thus not part of the AIS analysed (Table 22).

In nectar treated with PG + mould PE, PL, and Pectinex, the serum pectin was extensively degraded into two separate fractions, one of which was eluted in the void volume and was rich in neutral sugars, and the other which was eluted in the included volume contained relatively smaller amounts of neutral sugars (Fig. 35e, f, g). The fraction eluted in the void volume from nectar treated with PL represented 29.8% of the total amount of AGA and 72.8% of the total amount of neutral sugars. The corresponding values for this fraction from nectar treated with PG + mould PE, and Pectinex were 18.7% and 60.7%, and 28.1% and 75.9% respectively. These values should be considered in relation to the amount of AIS obtained after treatment with PG + mould PE (338 mg), PL (233 mg), and Pectinex (92 mg), as shown in Table 22. The presence of a fraction excluded from the gel in nectar treated with PG + mould PE, PL, and Pectinex suggests that the neutral sugar side chains may have a high degree of polymerization, because these sera gave sandy-like precipitates with alcohol, indicating that the main uronide backbone chain had a low degree of polymerization. Further support for this finding can be derived from the fact that the fragments in these sera did not precipitate with copper-ions when assayed for AGA concentration and DE (Section 3.2.14). Thus, it may be concluded that the pectic substances in apricots are characterized by the presence of regions without

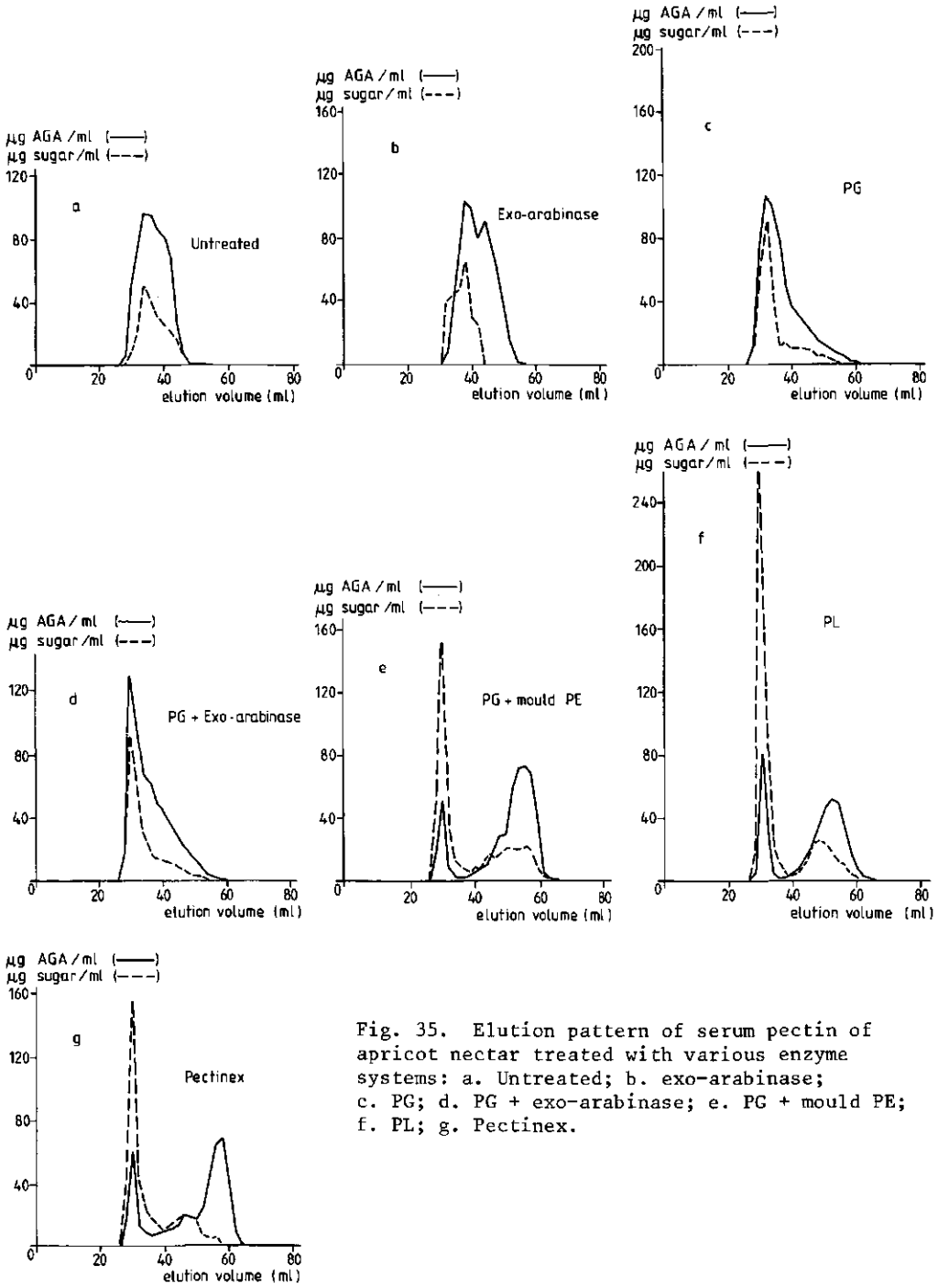


Fig. 35. Elution pattern of serum pectin of apricot nectar treated with various enzyme systems: a. Untreated; b. exo-arabinase; c. PG; d. PG + exo-arabinase; e. PG + mould PE; f. PL; g. Pectinex.

side chains (smooth regions), and regions containing high amount of neutral sugar side chains (hairy regions).

4.8 THE ROLE OF PECTIN FRACTIONS IN CLOUD LOSS AND GEL FORMATION IN APRICOT NECTAR

From the results discussed in the preceding sections, pectic substances in apricot puree would seem to play an important rôle in maintaining cloud stability. It appears that cloud stability can be achieved by degrading particular pectin fractions with specific enzymes. Therefore, further investigations were carried out in an endeavour to determine which fraction is responsible for the cloud loss and gel formation.

4.8.1 Sugar composition of pectic polysaccharides of apricot puree

Pectic substances of apricot puree were extracted sequentially with water, and solutions of ammonium oxalate, acid and alkali, and the residual material was fractionated into hemicellulose and cellulose as described in Section 3.2.5. The pectin fractions were found to constitute about 65% of the AIS. The water-soluble pectin (WS) and HCl-soluble pectin (HS) represented 40.3% and 37.4% respectively of the total pectin, and oxalate-soluble pectin (OS) 6.3%. Souty *et al.* (1981) found that WS and HS represented 41.0% and 55.0% respectively of the total pectin in fresh apricot (var. Rouge du Roussillon), and Navarro *et al.* (1982) found that these fractions represented 45.5% and 49.1% respectively of the total pectin in fresh apricot (var. Bulida). The difference between these values and those obtained in the present study may be attributed to the raw material used. In the canned apricots used in the present study, some of the protopectin may have been hydrolysed to water-soluble pectin.

The distribution of the AGA and neutral sugars over the various fractions is given in Table 25. The recovery of rhamnose was very low (72.8%) compared with that for other sugars. When the rhamnose values were calculated for 100% recovery, the pectin fractions contained 69.0% of the total amount of rhamnose present in the AIS. Most of it is present in the HS fraction (42.5%; 30.7% of the value actually found). Arabinose content of the AIS was present mainly in the pectin fractions (80%), particularly in the HS (60%), which also contained 38.5% of the total amount of AGA in the AIS. Thus, it would seem that this

Table 25. Distribution of sugars and uronide in the fraction of AIS (expressed as proportion of the total amount originally present in AIS of apricot puree)

Fractions	Neutral sugars						AGA
	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	
Pectin							
Water-soluble	9.0	10.4	8.8	12.1	18.6	2.7	43.7
Oxalate-soluble	3.5	1.9	1.1	1.9	2.7	3.5	6.3
HCl-soluble	30.7	59.1	9.0	18.2	19.7	0	38.5
NaOH-soluble	6.9	10.9	2.6	2.2	5.8	0.1	11.0
Hemicellulose	19.2	5.3	83.4	58.8	47.7	18.0	---
Cellulose	3.4	2.5	7.0	4.7	1.3	72.7	---
Total	72.6	90.0	111.9	97.9	95.8	97.1	99.5

fraction is rich in neutral sugars, particularly rhamnose and arabinose, and that it is similar to the high molecular weight fraction which is solubilized in the serum by cloud-stabilizing enzymes.

A very small amount of arabinose was found in the hemicellulose and cellulose fractions. Xylose, mannose, and galactose were present mainly in the hemicellulose fraction. Cellulose and hemicellulose contained 72.7% and 18.0% respectively of the total amount of glucose present in the AIS.

To ascertain whether the high amounts of neutral sugars in the HS were covalently linked to the galacturonan backbone, or whether they were derived from neutral polysaccharides contaminants, the fraction was subjected to ion-exchange chromatography. The elution patterns on a DEAE-cellulose column of WS, OS, and HS are shown in Fig. 36. The elution pattern of the HS fraction was different to that of the WS or OS fractions. Most of the neutral sugars in HS are shown to be linked to the pectin. The pectin fraction which is shown not to be bound to the column was found to contain 30% of the total amount of neutral sugars (Fig. 36). Thus, the HS pectin was saponified by dialysis against 0.1 N NaOH at 2 ^{o}C and subjected to ion-exchange chromatography on a DEAE-cellulose column. Only 5.2% of the total neutral sugars was found not to be bound to the column. The elution pattern is not shown because the recovery from the column was too low. The saponified uronides were bound firmly to the column and were difficult to elute even with 0.1 N NaOH. A similar observation was made by Rombouts & Thibault (1983) with saponified sugar beet pectin. Thus, it can be concluded that the neutral sugars in the HS are covalently linked to the pectin. Similar results were obtained by Souty *et al.* (1981).

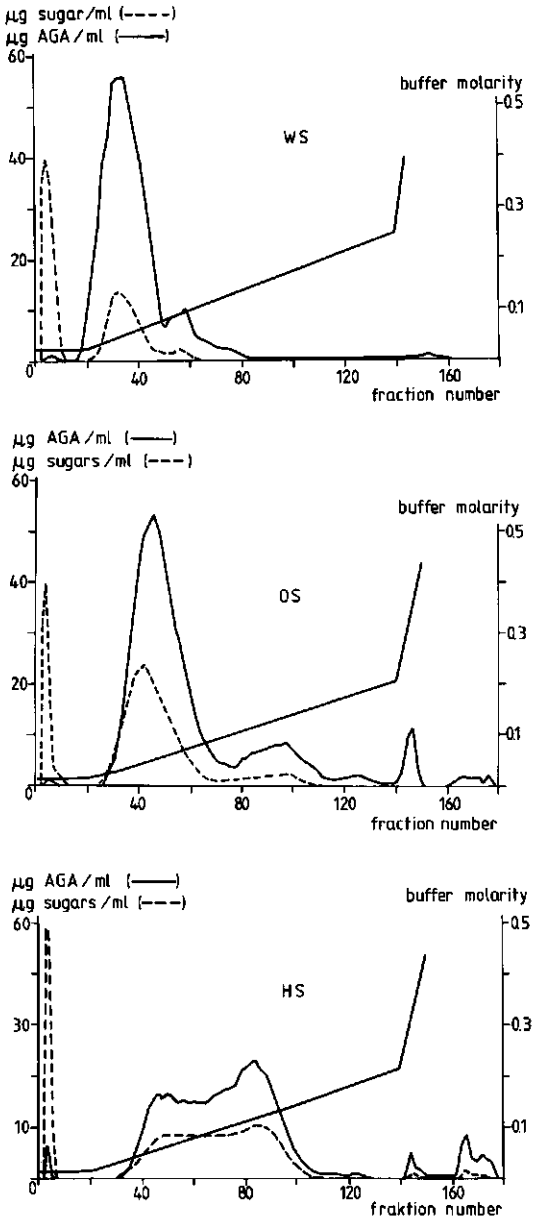


Fig. 36. Elution pattern of pectin fractions of apricot puree on DEAE-Cellulose: WS. Water-soluble pectin; OS. Oxalate-soluble pectin; HS. HCl-soluble pectin.

From the results presented in Sections 4.5, 4.6 and 4.7, it can be seen that the solubilization of uronides by the various pectolytic enzymes was accompanied by the solubilization of rhamnose, arabinose, and galactose. Cloud-stabilizing enzymes were found to release more rhamnose and arabinose than did non-stabilizing enzymes. Therefore, the rôle of the HS fraction in cloud loss and gel formation was examined.

4.8.2 Enzymatic degradation of HCl-soluble pectin

The HCl-soluble pectin fraction (HS) was treated with various pectolytic enzymes as described in Section 3.2.8, and the products of degradation examined by gel permeation chromatography on Sephacryl S-300. The elution pattern of the untreated pectin fraction is presented in Fig. 37a. The pectin was eluted as one peak in the void volume and the residues of neutral sugars were eluted in the same pattern as the galacturonide residues.

PG action. PG degraded the HS to a limited extent and split the pectin partly into two fractions (Fig. 37b). One fraction was eluted in the void volume and contained a higher amount of neutral sugars than the other fraction which was eluted in the included volume.

PG + mould PE action. HS was degraded more by the combined action of PG and mould PE than by the action of PG alone (Fig. 37c). The elution pattern shows that the pectin degraded into two separate fractions. One fraction was eluted in the void volume and contained 18.3% of the total amount of AGA and 94% of the total amount of neutral sugars. Of the total arabinose, 95% was in this fraction. This fraction consisted of rhamnogalacturonan with side chains of arabinan or arabinogalactan together with small amounts of xylose. The second fraction which contained 81.7% of the total amount of AGA was eluted in the included volume and can be considered to be a homogalacturonan. Thus, it can be concluded that the HCl-soluble pectin of apricot fruit is characterized by the presence of hairy regions.

PG + exo-arabinase action. Because of the high amount of arabinose in HS and the synergistic effect of PG and exo-arabinase discussed in Section 4.6, the effect of these two enzymes together on HS fraction was studied. The combined

action of these enzymes degraded this fraction into two separate fractions (Fig. 37d) similar to those produced by the combined action of PG and mould PE. The fraction eluted in the void volume contained 15.4% of the total neutral sugars, and 6.4% of the total amount of arabinose. However, arabinose constituted only 25.1% of the total amount of neutral sugars present in this fraction. The remainder was made up of: rhamnose, 41.3%; xylose, 4.0%; and galactose, 39.6%. The neutral sugar side chains especially arabinan were broken down by exo-arabinase, and the sugars liberated were eluted in the included volume. The action of exo-arabinase alone degraded the arabinan side chains into monomeric arabinose (Fig. 37e) as was shown by high performance liquid chromatography analysis of the included fraction. Thus, it would seem that the removal of the side chains made the pectin molecule accessible to PG action. The combined action of PG, PE and exo-arabinase resulted in only minor differences in the degradation profile obtained with PG + exo-arabinase (Fig. 37f). The pectin fraction in the void volume represents 12.9% and 17.8% respectively of the total AGA and neutral sugars.

PL action. PL activity degraded the HS fraction more than did the activity of PG (Fig. 37g). Since the DE of this fraction was 62.6%, it can be assumed that it was a better substrate for PL than for PG. Alternatively, the presence of a high amount of neutral sugar side chains may protect the fraction from PG activity but not from PL activity. Comparison of the elution patterns of the HS fraction degraded by PL action (Fig. 37g) and by PG + mould PE (Fig. 37c) shows that, even though both enzyme preparations acted on highly esterified pectin, PL gave a broad elution pattern, and PG + mould PE gave two distinct peaks. The fraction of HS treated with PL which was eluted from Sephacryl S-300 in the void volume (Fig. 37g) contained 11.6% of the total amount of AGA, 58.1% of the total amount of neutral sugars, and 54.5% of the total amount of arabinose. These values are much lower than those for combined action of PG and mould PE and indicate that highly esterified galacturonan segments located in the hairy regions are broken down more by PL activity than by the action of PG and mould PE. When PL was combined with exo-arabinase, the distribution of neutral sugars but not the galacturonides changed in the elution pattern (Fig. 37h). The amount of arabinose in the fraction eluted in the void volume decreased from 54.5% to 5.4% of the total of arabinose.

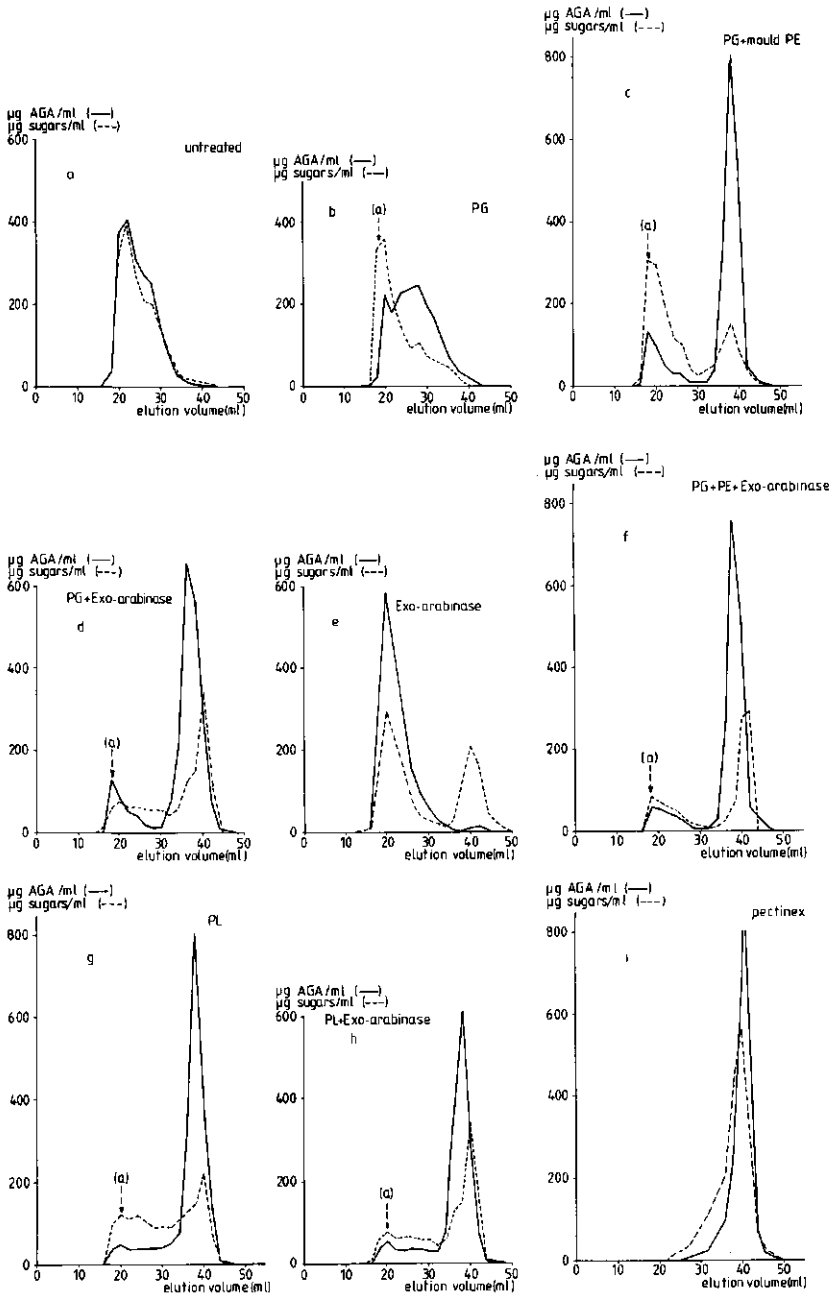


Fig. 37. Elution pattern from gel permeation chromatography of HCl-soluble pectin fraction of apricot puree degraded with various enzyme systems.
a. Untreated; b. PG; c. PG + mould PE; d. PG + exo-arabinase; e. exo-arabinase; f. PG + PE + exo-arabinase; g. PL; h. PL + exo-arabinase; i. Pectinex.

Pectinex action. Pectinex completely degraded the HS fraction (Fig. 37i). Because of the presence of a wide spectrum of polysaccharide-degrading enzymes, such as, arabinases, galactanases, xylanases, and glucanases, which break down the neutral sugar side chains, PG, PE and PL are able to degrade the galacturonan backbone completely. This supports the results obtained for PG + exo-arabinase action, that the neutral sugar side chains protect or prevent the pectin from being degraded by PG alone.

On the basis of these results, the definition is put forward that stabilizing enzymes are those which degrade the HCl-soluble pectin fraction in apricot tissue. Non-stabilizing enzymes degrade this fraction to a limited extent only. Therefore, it is concluded that the HCl-soluble pectin fraction plays an important rôle in the cloud stability of apricot nectar. If part of this fraction is solubilized from the cloud particles, no gel is formed. If sufficient is solubilized to rupture the cells, then the cloud particles are stabilized. This was confirmed by examination of cloud particles under the microscope.

4.9 COMPARISON OF COMMERCIAL AND LABORATORY-PREPARED APRICOT NECTARS

Four commercial apricot nectars (A, B, C, and D), two apricot nectars prepared in the laboratory (E and F) were analysed visually, microscopically and chemically. Only the commercial nectar B and the laboratory-prepared nectar F were cloud stable throughout the experimental period (Fig. 38). Nectar B contained stabilizing agents as indicated on the label. Nectar F contained a cloud-stabilizing enzyme. Rapid cloud loss occurred in commercial nectar D; when the nectar was left to stand a clear serum formed on the top. Cloud loss in commercial nectar C occurred in two stages; initially the serum was turbid but after three weeks it became clear. Commercial nectar A was more stable than nectars C, D, and E.

The cloud particles predominating in each nectar were identified by microscopy (Fig. 39). Nectars D and E contained mainly intact cells with thick cell walls, while nectars A, B, C, and F contained degraded cells and cell wall fragments. The difference in the shape of the cloud particles in the commercial nectars is due to the extent of homogenization. If the nectar was homogenized

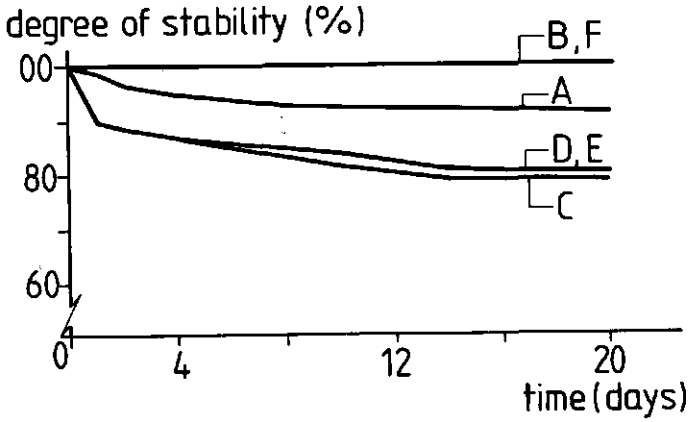


Fig. 38. Cloud stability of commercial and laboratory-prepared apricot nectars: A, B, C, and D. commercial nectars; E. Untreated, laboratory-prepared nectar; F. laboratory-prepared nectar treated with stabilizing enzyme.

to such an extent that the cells were ruptured and cell wall fragments were formed, cloud stability was improved but not totally. A weak gel formed in some nectars after sedimentation. Thus, homogenization alone did not achieve cloud stability, stabilizing agents had to be added, as in nectar B. The amount of neutral sugars and AGA in cloud particle AIS and the DE of the insoluble pectin of commercial and laboratory-prepared nectars are presented in Table 26.

Table 26. Polysaccharide composition of cloud particles of commercial and laboratory-prepared nectars (All weights are expressed as mg/100 ml nectar.)

	Type of nectar*					
	A	B**	C	D	E	F**
Weight of AIS	280	540	380	384	436	270
AGA	23.6	67.1	30.9	23.2	92.4	7.3
Neutral sugars						
Rhamnose	5.5	10.3	6.8	8.1	16.5	5.2
Arabinose	12.8	23.2	12.4	15.1	37.6	6.8
Xylose	13.0	31.9	17.9	27.3	17.3	15.2
Mannose	9.5	16.9	13.0	13.4	15.3	14.0
Galactose	12.3	26.5	17.0	18.3	23.7	15.0
Glucose	89.9	168.2	118.0	142.0	131.1	123.3
DE %	63.0	35.9	57.0	46.2	63.2	19.7

* A, B, C, and D are commercial nectars; E. laboratory-prepared nectar; and F, laboratory-prepared nectar treated with a cloud stabilizing enzyme.

** Cloud-stable nectar.

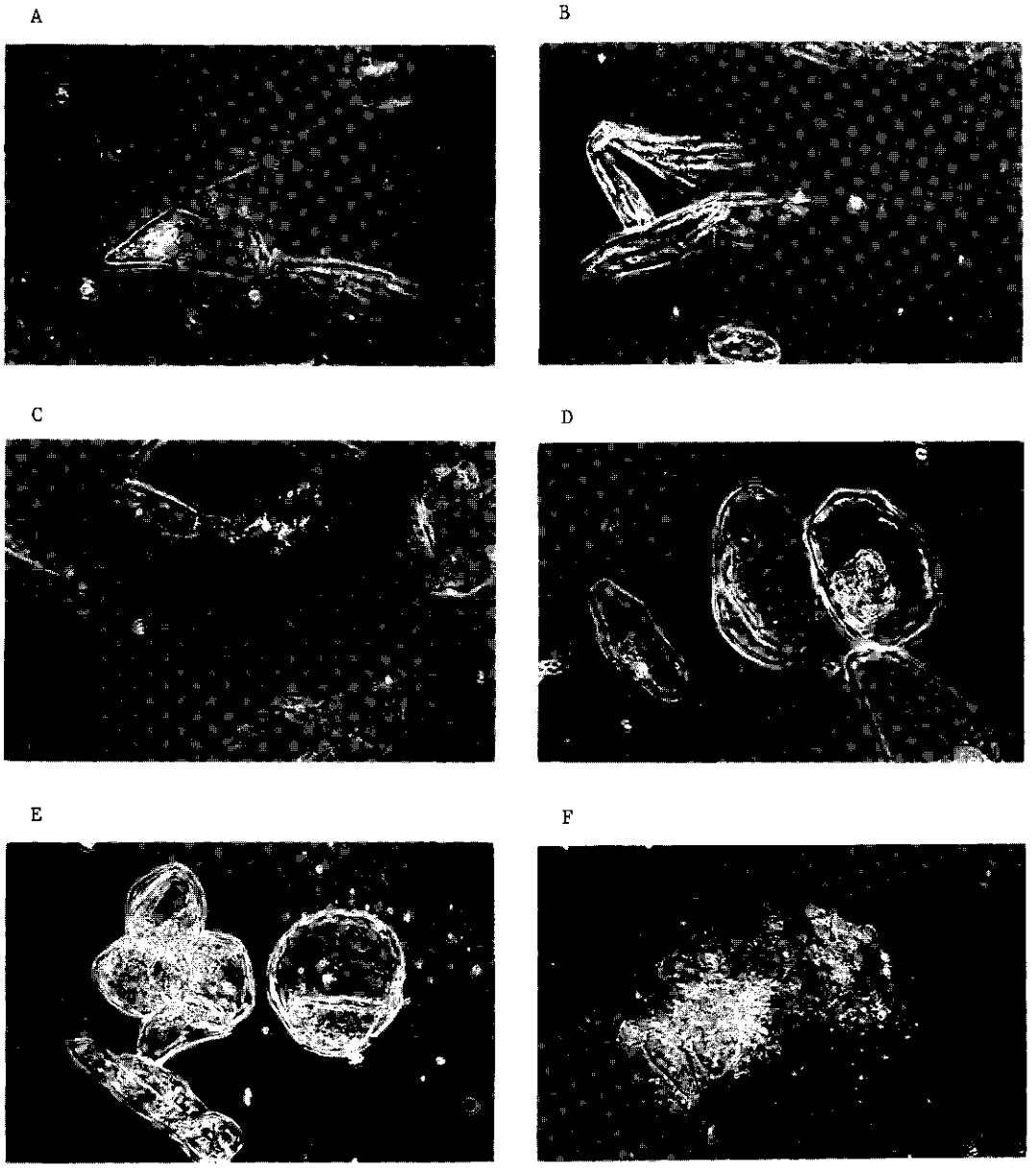


Fig. 39. Cloud particles of commercial and laboratory-prepared nectars: A, B, C, and D are commercial nectars; E laboratory-prepared nectar, and F laboratory-prepared nectar treated with a cloud stabilizing enzyme.

The differences in the weight of AIS and sugar composition are due to the raw material used (fresh apricots, canned apricots, or puree); the fruit itself (variety and stage of ripening); and manufacturing process (heat treatment and homogenization). There was no distinct difference between nectar B and nectars A, C, D and E. The type of stabilizing agent added to nectar B cannot be identified from the data presented in Table 26, because the stabilizer is present in the serum. Nectar F was quite different to the other nectars. Due to the enzymatic action of the cloud-stabilizing enzyme, the cell walls of the cloud particles were attacked, and the cells were ruptured (Fig. 39). Thus, the amounts of AGA and arabinose and also the DE were lower in nectar F than in the other nectars (Table 26). This indicates that the pectin fraction rich in neutral sugar side chains, especially arabinan, was removed from the cloud particles. This fraction is considered to be responsible for cloud loss and gel formation.

4.10 EFFECT OF PECTOLYTIC ENZYMES ON DENSITY OF CLOUD PARTICLES AND FLOW BEHAVIOUR OF APRICOT NECTAR

4.10.1 Effect on the density of the cloud particles

According to Stokes' law (Section 2.6), the density of the suspended particles affects the rate of sedimentation. This is only valid for a suspension in which the suspended phase does not interact with the suspending medium and the suspended particles are spherical. Further, the validity of Stokes' law is confined to Reynolds numbers (Re) between 10^{-4} and 1 approximately

$$Re = \nu \rho_c d/\eta_c$$

where

ν = sedimentation rate of the particles ($m.s^{-1}$)
ρ_c = density of the continuous phase ($kg.m^{-3}$)
d = diameter of the particles (m)
η_c = viscosity of the continuous phase ($P_a.s$).

At $Re < 10^{-4}$ particles do not show steady sedimentation because of brownian movement and at $Re > 1$ laminar flow changes into turbulent flow. Therefore, the formula should be used with caution to explain sedimentation behaviour in

fruit nectars. Nevertheless, if serum viscosity does not play a rôle in the stability of fruit nectars (as described in Section 2.5.2 and Section 4.4), and if the particles density decreases, based on Stokes' law, cloud stability of the nectar will be improved.

The addition of pectolytic enzymes to apricot nectar has been shown to result in a reduction in cloud particle diameter, because cell aggregates or cell clusters are disintegrated into individual cells. Moreover, the stabilizing enzymes attacked the cell wall and destroyed the cells, thus producing particles of smaller diameters. Consequently, d (diameter) in Stokes' law is decreased, and the decrease in ρ_s (density of the particles) will contribute to a slower rate of sedimentation. Because of the high osmotic pressure of sugar solutions used to establish the gradients, the density distribution shown in Table 27 may not represent the true density, and therefore, it is referred to as the apparent density.

Table 27. Effect of Rohament P and Pectinex on apparent density distribution of the cloud particles of apricot nectar (expressed as proportion of weight %.)

Apparent density $kg.m^{-3}$	Untreated	Pectinex added	Rohament P added
1.203	-	6.7	-
1.260	1.8	7.7	8.4
1.319	10.5	50.6	50.4
>1.319	87.8	35.2	41.3

The effect of Rohament P and Pectinex on the apparent density distribution of the cloud particles is shown in Table 27. Addition of Rohament P or Pectinex resulted in the release of cell wall components. Nectar treated with Rohament P contained a much larger fraction of cloud particles of low density than untreated nectar. This could explain why cloud stability was improved with the addition of Rohament P. Nectar treated with Pectinex contained even a larger fraction of particles of low density than nectar treated with Rohament P. This is in agreement with the changes in cloud particle weight (Sections 4.5.2 and 4.5.4). Pectinex solubilized 46% and Rohament P solubilized 40% of cloud particles. The changes in particle density distribution are also in accordance with the stabilizing effect achieved by Pectinex and by Rohament P. Pectinex stabilized cloud particles completely, but Rohament P only improved the level

of cloud stability.

4.10.2 Flow behaviour of apricot nectar

In the previous sections, cloud stability has been shown to be related to the action of specific enzyme systems. However, a better understanding of this phenomenon can be obtained by relating cloud stability to changes in flow behaviour. Untreated apricot nectar showed almost a plastic flow (non-Newtonian) with an estimated yield stress of 0.24 P_a (Fig. 40). For nectars treated with enzymes, pseudoplastic flow with higher yield stress was observed. The viscosity above the yield stress was lower for treated than for untreated nectar. The yield stress increased from 0.24 P_a for untreated nectar to 0.44 P_a and 1.02 P_a for nectar treated for 3 hr with Rohament P and Pectinex respectively.

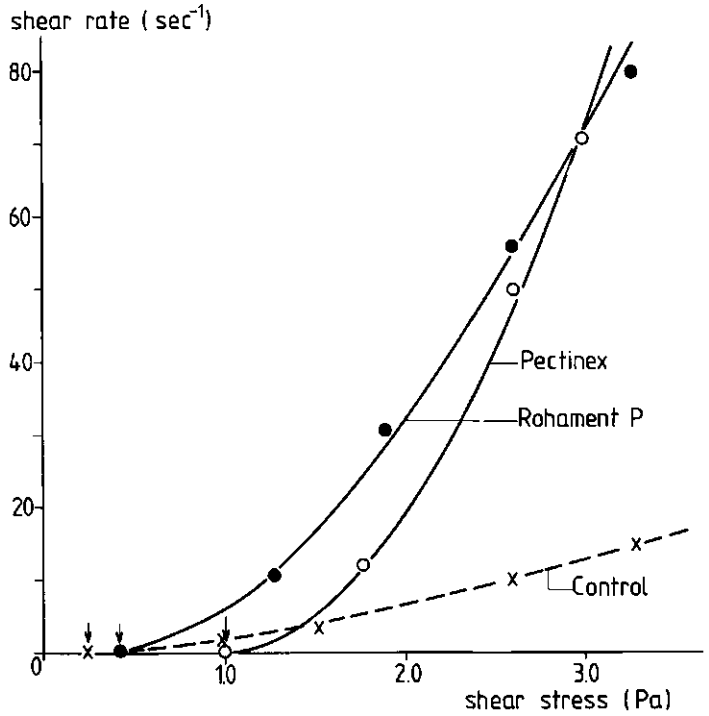


Fig. 40. Flow behaviour of apricot nectar:
(x—x) untreated; (•—•) treated with Rohament P;
(o—o) treated with Pectinex.

The yield stress was highest in the nectar treated with Pectinex, which completely stabilized the cloud particles, and was lower in nectar treated with enzyme preparations which improved cloud stability to a lesser extent.

Serum of untreated and treated nectar showed a Newtonian flow, but the viscosity of serum of untreated nectar was higher than that of serum of treated nectar. The viscosity of serum of Pectinex-treated nectar decreased faster and to a greater extent than that treated with Rohament P (Fig. 41). This is in accordance with the distribution of molecular weight of serum pectin (Sections 4.5.2 and 4.5.4). Thus, it can be concluded that cloud particles are responsible for the non-Newtonian behaviour of the nectar and that polymers in the serum do not contribute to yield stress. The same has also been reported for apricot puree by Costell & Duran (1979) and for orange juice by van Vliet & van Hooijdonk (1984).

Technologically, fruit nectars differ from fruit puree because they are mixed with other ingredients, homogenized, de-aerated, and pasteurized. Furthermore, cloud particles and serum of nectar treated with enzymes differ chemically from those of untreated nectar. Therefore, the increment in yield stress as a result of enzyme action can be attributed to disintegration of the cloud particles and particularly to modification of the cell walls. It is speculated that, due to the release of some of the pectin from the cell walls by Rohament P, the hydration capacity of the cellulose microfibrils is increased, thus resulting in an increase in the yield stress. However,

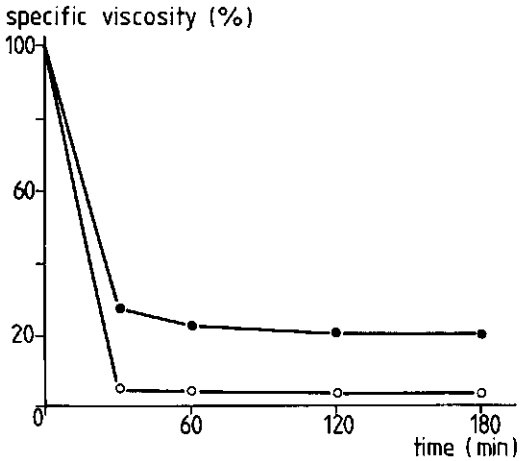


Fig. 41. Effect of Rohament P (•—•) and Pectinex (o—o) on serum viscosity of apricot nectar.

hydration capacity and yield stress are higher when sufficient pectin is released from the cell walls to liberate more cellulose microfibrils from the enclosing material (wide spectrum pectolytic enzymes, such as, Pectinex).

It is conceivable that hydration of the cellulose microfibrils allows more interaction between the cloud particles and between cloud particles and components in the serum, thus increasing the suspending properties of the cloud particles. Such interactions increase the yield stress in the nectar. When these interactions are destroyed as a result of high shear stress, the viscosity decreases below that of untreated nectar (Fig. 40).

5 General discussion and conclusions

Unusual cloud-loss behaviour was observed in pasteurized apricot nectar which was prepared by mixing apricot puree with an aqueous sugar solution. When the mixture was left to stand, cloud particles settled slowly, but during this process a gel formed. Similar behaviour has also been observed in pasteurized passion fruit nectar. In pasteurized orange juice, cloud particles remain regularly dispersed. This has been ascribed by van Vliet & van Hooijdonk (1984) to the formation of a weak gel when the juice is left to stand. In pasteurized orange juice, cloud loss can be induced with the addition of PE, but when PE is added in the presence of a calcium chelating agent, cloud loss does not occur. In this study, it was found that cloud loss in apricot nectar was neither prevented by calcium chelation nor enhanced by the addition of PE.

Cloud-loss behaviour was also found to be independent of serum pectin. However, cloud was stabilized when cell walls were disintegrated by specific enzyme action.

The data obtained on the polysaccharide composition of apricot cell wall material indicate that the pectic substances are rich in neutral sugars. The main neutral sugars found in pectin fractions from apricot puree AIS were rhamnose, arabinose, and galactose. The distribution of these sugars in the various pectin fractions was found to differ. The water-soluble pectin fraction contained only small amounts of neutral sugars with similar amounts of arabinose and galactose. The HCl-soluble pectin fraction contained higher amounts of neutral sugars, especially arabinose. Gel permeation and ion-exchange chromatography revealed that the neutral sugars were linked to the rhamnogalacturonan backbone of the HCl-soluble pectin. Rhamnose has been shown to be an integral part of the chain, and arabinose and galactose are present as side chains (Talmadge *et al.*, 1973; Albersheim, 1975; Knee *et al.*, 1975; de Vries *et al.*, 1982).

The side chains were shown to be present mainly in hairy regions with sections of homogalacturonan in between. This indicates that the structure of apricot pectin is similar to that of apple pectin (de Vries *et al.*, 1983) and of cherry pectin (Thibault, 1983). However, the side chains in apricot pectin were shown to consist mainly of arabinose with a very little amount of

galactose. Acidic polysaccharide fragments rich in arabinose have also been isolated from vegetable and fruit tissues, for example, pear (Ahmed & Labavitch, 1980), carrot (Stevens & Selvendran, 1984), apple (Fogarty & Fanous, 1984), and cabbage (Stevens & Selvendran, 1980). On the other hand, pectic substances isolated from apple (de Vries *et al.*, 1983), tomato (Gross & Wallner, 1979; R. Heutink, unpublished data) and potato (Ishii, 1978) have been characterized as galactose-rich acidic polysaccharides. More detailed study on the structure of the side chains of apricot pectin was not within the scope of the present investigation.

The results of this study indicate that the hairy regions play an important role in controlling the mode of action of some pectolytic enzymes. Mould PE de-esterified soluble and insoluble pectin fractions to a greater extent when it was present in a wide spectrum enzyme preparation (Pectinex) than in purified form alone. Thus, it may be concluded that the neutral sugar side chains protect the methylgalacturonides of the hairy regions and that these side chains are removed by the hemicellulases in the wide spectrum preparation. The PL contained in the wide spectrum preparation can attack hairy regions and release pieces of polygalacturonide in between, which are good substrate for PE.

Pure PG or commercial PG preparations, such as Rohament P, were found not to be able to degrade completely pectins having many hairy regions, such as the HCl-soluble pectin fraction. Both the hairy regions and the methoxyl groups of apricot pectin impede the activity of PG. If the pectin is de-esterified by simultaneous action of PE, or if arabinan side chains are removed by simultaneous action of arabinase, more extensive break-down of pectin can be observed. This is in agreement with the findings of Ahmed & Labavitch (1980) who have suggested that pectic arabinan in pear fruit protects the backbone from PG action. They assumed that the component containing the arabinosyl residue consists of a series of short segments, which are spaced at close, regular intervals along the backbone. Also, galactose-rich polysaccharide regions in tomato cell walls have been shown to restrict the action of tomato PG (Gross & Wallner, 1979).

PL has been shown to attack the hairy regions and to degrade the pectin molecule into small fragments soluble in 80% ethanol. This is in agreement with results obtained by Ishii (1976) who reported that 70% to 80% galacturonides released by PL from onion cell walls was present in the ethanol-soluble form, but PG only formed 10% of the alcohol soluble fraction. Ishii (1978) also found that higher amounts of neutral sugars were released from potato cell walls by

by endo-PL than that by endo-PG.

The enzyme systems which were found to degrade cell wall pectin of apricot nectar, particularly the HCl-soluble pectin fraction are: PG + mould PE; PG + exo-arabinase; PL; and wide spectrum commercial pectinases, such as Pectinex. These have been defined as stabilizing enzymes. Examination of cloud-stable apricot nectar under the microscope showed that cloud stability had been achieved in tissue in which the cell walls were at least partly degraded so that the cells were ruptured. This effect was only achieved with the enzyme system that degraded the HCl-soluble pectin fraction to a great extent. Non-stabilizing enzymes (commercial PG preparations and purified PG) degraded this fraction to a limited extent only. Further, the viscosity of serum of cloud-stable nectar was similar to that of a sugar solution of the same Brix value, thus indicating that the serum pectin had been completely depolymerized. Similar results were obtained by Weiss & Sämann (1972). These enzyme systems have also been demonstrated to be well suited for fruit pulp-enzyming (Voragen & Pilnik, 1981) where their effect goes beyond maceration which sometimes is used to obtain a cloud-stable cell suspension in a viscous serum (Grampp, 1969; Sulc & Vujicic, 1973; Strübi, 1976; Schmitt, 1980). However, it is doubtful whether macerating enzyme systems can achieve cloud stability. Zetelaki-Horvath & Gatai (1977) have shown that vegetable tissue (e.g., celery, pepper, and carrot) treated with pure PG do not yield cloud-stable juice but the combined action of mould PG and ultrasonication gave cloud-stable juice. Moreover, the manufacturers of Rohament P, who describe this almost pure PG as a macerating enzyme, recommend the use of mechanical maceration (homogenization) together with Rohament P to obtain cloud-stable products. Bock *et al.* (1973) and Shomer *et al.* (1984) demonstrated that increasing serum viscosity by adding pectin did not prevent settling of tomato particulates, which is also contrary to the theory that maceration is responsible for cloud stability.

Rheological measurements carried out in this study indicate that modification of apricot cell walls by stabilizing enzymes gives the nectar a higher yield stress and reduces the apparent cloud-particle density. This is mainly due to the release of pectic polysaccharides which make up most of the weight of the cloud particles. Probably, the cellulose microfibrils liberated became more hydrated and interacted with constituents in the serum and the resultant structure was responsible for the increased yield stress and the improved cloud stability. A similar phenomenon has been reported by Shomer *et al.* (1984) who found that pectinase treatment of tomato juice induced swelling of the

cellulose and improved the suspending properties of the particulates. Whittenberger & Nutting (1958) have shown that the release of electrolytes including soluble pectin, organic acids, and mineral salts led to swelling of the walls of tomato juice particles which became increasingly hydrophilic, thus increasing viscosity. Homogenization destroyed the spheroid shape of the cellulose shells and shredded the walls into twisted ribbons and irregular sheets, thus suggesting that the increased viscosity is the result of these changes in the structure.

From the results of this study, the stabilization mechanism in apricot nectar may be described as follows. Gel formation and clarification of apricot nectar are affected by the pectin fraction rich in neutral sugars, especially arabinose. This pectin fraction is a structural element of cell walls. If part of the fraction is solubilized, cells separate resulting in separate, intact cells with thinner cell walls. Thus, gel formation is prevented and the level of cloud stability improved. If solubilization increases to such an extent that part of the cell walls is ruptured, the particles became lighter and more hydrated. The resultant structure gives the nectar a high yield stress and improves cloud stability.

Thus, apricot nectar may be stabilized in various ways. Firstly, this may be achieved by intensive mechanical treatment of the nectar with suitable equipment, such as colloidal mills, to rupture the cells together with the addition of appropriate stabilizing agents. Commercial cloud-stable nectars made in this way are usually labelled to contain stabilizing agent. Secondly, stabilizing enzymes may be used. This is more desirable, as this avoids the legal problem about the use of stabilizers and also saves on the energy required for intensive homogenization. The greatly reduced serum viscosity permits concentration of fruit puree, thus reducing the cost of transport and storage. The increasing use of commercial pectolytic enzymes in the manufacture of fruit and vegetable products may assist in maintaining the costs of these enzymes for use in cloud stabilization of fruit nectars at a reasonable low level. Thirdly, there is the combination of mechanical and enzymatic treatment, which is, in fact, recommended by some enzyme manufacturers. The decision on which process to use will also depend on the sensory properties of the nectar. However, evaluation of these properties was not within the scope of this study.

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