# Tomato juices and tomato juice concentrates: a study of factors contributing to their gross viscosity

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 8 oktober 1986
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

**STELLINGEN**

1. De door Shomer et al. (1984) gesuggereerde rol van endogeen cellulase bij het uitzakken van deeltjes in tomatensap wordt niet ondersteund door de in de literatuur vermelde cellulase hoeveelheden in de tomaat.

   Shomer, I., P. Lindner en R. Vasiliver, 1984
   Journal of Food Science, 49: 628–633

2. Gezien de grootte van de deeltjes is het twijfelachtig of Rao et al. (1986) de juiste meetgeometrie gebruiken voor hun rheologische metingen aan appelmoes.

   Rao, M.A., H.J. Cooley, J.N. Nogueira en M.R. McLellan, 1986
   Journal of Food Science, 51: 176–179

3. Bij het gebruik van polysaccharide-afbrekende enzymen ter opheldering van de structuur van polysacchariden moet meer aandacht worden besteed aan:
   1) de zuiverheid van het enzym, 2) de zuiverheid van de substraten die worden gebruikt voor het karakteriseren van het enzym en 3) een mogelijke multisubstraat specificiteit van het enzym.

4. De vorming van hydroxymethylfurfural is geen bruikbare indicator voor het optreden van niet-enzymatische bruinkleuring in vruchtesappen.

5. Alleen door het geven van uitgebreide voorlichting kan worden bereikt dat de consument bestraling van voedingsmiddelen als een alternatieve conserveringsmethode aanvaardt. Het effect van een voorlichtingscampagne zal worden tenietgedaan wanneer dit proces in verhullende benamingen op het produkt wordt gedeclareerd.

6. Het zich toeëigenen van het woord significant door statistici kan tot verwarring leiden wanneer dit woord in het Engels wordt gebruikt in de betekenis van veelbetekenend.

7. De viscositeit van tomatensap en concentraten daarvan wordt grotendeels bepaald door het gehalte aan wateronoplosbare bestanddelen.

   Dit proefschrift

9. Een enzymatische hydrolyse met een mengsel van pectolytische en cellulolytische enzymen, voorafgaand aan een chemische hydrolyse met 2N TFA is noodzakelijk om de neutrale suikersamenstelling in tomaten WIS te kunnen bepalen.

   Dit proefschrift

10. Het is mogelijk om tomatensap te concentreren zonder dat na verdunning van het concentraat een verlies in viscositeit ten opzichte van het ongeconcentreerde sap zal optreden.

Dit proefschrift

11. Verschillen in viscositeit tussen hot break en cold break tomatensap worden niet alleen veroorzaakt door de snelheid van enzym-inactivatie maar ook door de temperatuur tijdens het passeren.

Dit proefschrift

12. Het gebruik van microscopische technieken om veranderingen in voedingsmiddelen vast te stellen kan gemakkelijk leiden tot "wishful seeing" als variant van "wishful thinking".

*Abstract*

R. Heutink, 1985. Tomato juices and tomato juice concentrates: a study of factors contributing to their gross viscosity. Doctoral Thesis, Agricultural University, Wageningen, The Netherlands.

The gross viscosity of tomato juice and tomato juice concentrates was found to be determined primarily by the water insoluble solids (WIS) content. The serum viscosity did not contribute to gross viscosity. The WIS consisted of whole tomato cells, vascular bundles and skin fragments. In general the WIS could be fractionated into 40-45% pectin, 25-30% hemicellulose and 30-35% cellulose. Highly branched as well as more linear pectin fragments were found to be present in tomato WIS. Xylans, arabinans and (arabino)galactans were involved in the attachment of pectic substances to the cell wall matrix. The presence of xylans, xyloglucans, (gluco)mannans and a limited amount of galactan in the hemicellulose was indicated. Pectin fragments, characterized by a low content of arabinose and galactose containing side chains, and esterified in such a way that they were degraded by both PG and PL, made an important contribution to gross viscosity. Cellulose was found to make a relatively smaller contribution to gross viscosity than these pectin fragments. The rigidity of the cell walls, caused by the cellulosic structure, seemed to influence gross viscosity at higher WIS concentrations. The differences which exist naturally in WIS composition were found to be too small to cause large differences in gross viscosity. Higher hot break temperatures were found to result in a more effective removal of WIS from skin material. The influences of hot break temperature, finisher temperature and size of finisher screen opening on the finisher operation were closely related to the efficiency of the finisher. The concentration of a tomato juice by evaporation resulted in a loss in gross viscosity, after dilution of the paste to the original strength. The causes for this "dilution-loss" were studied and could be related to the simultaneous concentration of tomato cells and, presumably, ions. The centrifugation-serum-concentration method resulted in a significantly decreased "dilution-loss".

*Curriculum vitae*

Rob Heutink was born in Enschede, the Netherlands, on the 29th of May 1956. From 1974 to 1980 he studied food science and technology at the Agricultural University of Wageningen, the Netherlands. In 1980 he obtained the degree of Master of Science, cum laude, with majors in food chemistry and food microbiology and with a minor in toxicology. From October 1980 to May 1985 he undertook, in the Department of Food Science of the Agricultural University, Wageningen, the H.J. Heinz Co. funded research work presented in this thesis.

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*SUMMARY*

The subject and objectives of the present study are introduced in chapter 1.

The relevant data from the literature are discussed in chapter 2. This literature review covers topics such as: the production, general composition and processing of tomatoes (2.1.); definitions and quality parameters for tomato products (2.2.); factors contributing to the gross viscosity of tomato products (2.3.); factors affecting serum separation in tomato products (2.4.) and the composition of tomato polysaccharides (2.5.). For convenience the lengthy and extensive review on the factors contributing to gross viscosity of tomato products is summarized in 2.3.1.

The general analytical methods used throughout this study are presented in chapter 3. Special attention has been paid to a comparison of two methods of measuring gross viscosity and to the determination of the neutral sugars present in tomato WIS. It was found that the Bostwick consistometer is not sensitive enough for measuring high-viscosity tomato products. This instrument, however, is very suitable for measuring the gross viscosity of tomato products with a Bostwick flow of 5 cm or more. The hydrolysis of the polysaccharides in tomato WIS with 0.8 N $H_2SO_4$ or 2 N $H_2SO_4$, both after prehydrolysis with 72% $H_2SO_4$, did not result in an adequate determination of the neutral sugars. Prehydrolysis of the polysaccharides with an enzyme mixture containing pectolytic and cellulolytic enzymes, prior to hydrolysis with 2N TFA, increased in particular the amounts of glucose which were determined.

The influence of soluble solids (serum viscosity) and insoluble solids on the gross viscosity of tomato juice and concentrates is studied and discussed in chapter 4. The samples analysed differed in variety and methods of processing (break temperature and concentration). The gross viscosity of tomato juices and concentrates was found to be determined primarily by their WIS content. Variety and method of processing influenced gross viscosity at a specific °Bx by affecting the amount of WIS. Gross viscosity at a specific WIS amount was not influenced by variety, serum viscosity or method of processing unless the method of processing resulted in degradation of the WIS.

In chapter 5 the results of studies on the composition and structural

features of tomato WIS are presented and discussed. WIS fragments, solubilized from WIS with solvents like oxalate, HCl and NaOH or by using purified enzymes, were characterized by gel permeation and ion exchange chromatography and by analysis of neutral sugar and glycosidic linkage composition. In general tomato WIS could be fractionated into 40-45% of pectin, 25-30% of hemicellulose and 30-35% of cellulose.

The pectin fraction contained large amounts of AGA and protein and moreover approximately 25-35% of the arabinose and galactose present in the WIS. Most of this arabinose and galactose was found in the HCl soluble pectin and formed part of a highly branched pectin segment solely linked to the cell wall matrix through highly esterified AGA chains.

A large proportion of the pectin occurred as fragments with a low content of arabinose and galactose side chains. These fragments were esterified in such a way that they were susceptible to both PG and PL action.

Xylans, arabinans and (arabino)galactans were found to be involved in the attachment of pectic substances to the cell wall matrix.

The tomato varieties which were studied, differed mainly in the WIS amount and in the arabinose and galactose content of this WIS. This difference was most clearly expressed in the HCl soluble pectin fraction. The composition of this fraction was found to change when tomatoes were processed into juice and paste.

The tomato hemicellulose contained approximately 80% of the WIS-xylose. 60% of this amount was present in β-1,4-xylans which are probably bound to pectin. 30% of the hemicellulose xylose was found to be present in fragments with a composition typical for xyloglucans. The hemicellulose also contained ± 50% of the arabinose and galactose present in the WIS and 15-20% of the WIS-glucose. All the arabinose and the major part of the galactose found in hemicellulose were present as terminal residues. The presence of (gluco)mannans and a limited amount of galactan in the hemicellulose fraction was indicated.

The cellulose fraction consisted mainly of glucose but also contained ± 15% of the arabinose present in WIS.

The results of studies on the relative contribution of WIS constituents to the gross viscosity of tomato juices and tomato juice concentrates are presented and discussed in chapter 6. Technical as well as purified, well characterized enzymes were used to decrease the gross

viscosity of a diluted tomato paste and of a 10°Bx tomato juice concentrate. All results, irrespective of the tomato product used, pointed to the important contribution of the more highly esterified parts of the WIS pectin to gross viscosity. It was considered likely that these parts of the pectin were identical to the parts susceptible to degradation by both PG and PL, as described in chapter 5. The importance of the low esterified pectin and the hemicellulose as contributors to gross viscosity remained unclear. The role of the protein present in WIS as a contributor to gross viscosity was found to be negligible. Cellulose was found to make a relatively smaller contribution to gross viscosity than esterified pectin. The rigidity of the cell walls caused by the presence of cellulosic structures, seemed to influence gross viscosity at higher WIS concentration.

It was concluded that the small differences in WIS composition, which occurred naturally in the tomato varieties which were studied, cannot cause large differences in gross viscosity. The gross viscosity of tomato juices and tomato juice concentrates therefore seemed to be determined primarily by the amount of WIS, which is probably proportional to the amount of tomato cells.

In chapter 7 the influence of processing factors such as hot break temperature, finishing operation, concentration and pasteurization on the WIS characteristics and gross viscosity of tomato juice and concentrates is discussed.

The increase in the gross viscosity of tomato juices and tomato juice concentrates as a result of an increase in hot break temperature was attributed to a more effective removal of WIS from the skin material. WIS "quality" and the microscopic appearence of the tomato cells were not affected by higher hot break temperatures. The influences of hot break temperature, finisher temperature and size of finisher screen opening on the finisher operation were found to be closely related to the effectiveness of the finisher.

Neither the method of concentration nor the type of evaporator used for concentration had an influence on the gross viscosity of concentrates. In general the WIS/TS ratio was found to remain fairly constant during concentration. A "dilution-loss" was observed when certain serum components (probably ions) were concentrated by evaporation in the presence of the

tomato cells. The magnitude of the "dilution-loss" was found to be influenced by mechanical shear, vacuum and heat processing of the concentrated juice. Hot break temperature and temperature of evaporation had no influence on the magnitude of the "dilution-loss". The quality of the juice before concentration also influenced the "dilution-loss". The centrifugation-serum-concentration technique was found to prevent "dilution-loss" when a bench top centrifuge was used and to reduce "dilution-loss" when a decanter was used. Both heat processing and freezing were found to decrease the gross viscosity of tomato juice and concentrates.

## *LIST OF ABBREVIATIONS*

AGA = anhydrogalacturonic acid

AIS = alcohol insoluble solids

Ara. = arabinose

DE = degree of esterification of AGA (%)

DEAE = diethylamino-ethyl

EDTA = ethylenediaminetetra-acetate

g = acceleration due to gravity ($m.s^{-2}$)

Gal. = galactose

glc = gas liquid chromatography

glc-ms = combined gas liquid chromatography and mass spectrometry

Gluc. = glucose

HPLC = high pressure liquid chromatography

Man. = mannose

MW = molecular weight

NS = neutral sugar

n.s. = not significant

PE = pectinesterase

PG = polygalacturonase

PL = pectinlyase

pnp = para-nitrophenyl

Rham. = rhamnose

TFA = trifluoracetic acid

WIS = water insoluble solids

WIS-ara. = arabinose present in WIS. In the same way WIS-AGA etc.

Xyl. = xylose

$\eta_{app}$ = gross viscosity measured with the Haake rotovisco and expressed as apparent viscosity (mPa.s).

$\eta_{serum}$ = serum viscosity

*CONTENTS*

# 1. INTRODUCTION

The tomato, a member of the Solanaceae family and therefore related to the nightshades, was not generally accepted as a food item until the middle of the 19th century.
Since then, the world production of tomatoes for consumption has increased and in 1979 it ranked third in the world production of fruits (grapes and citrus fruits ranking first and second). The nutrient value of the tomato is not particularly high but because of the large consumption the tomato stands first in the total contribution of a group of 10 vitamins and minerals in the US diet (Rick 1978). Approximately 70-80% of the total annual tomato crop is used in the tomato processing industry. In Europe tomato paste is the main tomato product (± 70% of the total processed tomatoes). Other products are: peeled tomatoes (21%); passata, a slightly concentrated tomato juice (6%); and tomato juice (2%).
Secondary tomato products such as ketchup and sauces are produced either from fresh tomato juice or from tomato paste.

An important characteristic of tomato products is gross viscosity. Gross viscosity describes the result of a viscosity measurement of the whole tomato product, in contrast to serum viscosity which describes the viscosity of the tomato product freed of the suspended particles.
It is well known that tomato juices and pastes of high gross viscosity are obtained when tomatoes are heated to a temperature exceding 90°C (hot break) before the removal of the skins and seeds. Juices and pastes of low gross viscosity are obtained when tomatoes are heated to 40-60°C (cold break) before the removal of skins and seeds. This difference has always been ascribed to the degradation of pectin by pectolytic enzymes. The literature, however, shows no agreement on the contribution of serum viscosity and water insoluble solids constituents such as pectin, cellulose and protein, to gross viscosity. No exact data are available on the composition and structure of the polysaccharides present in tomato products and on the relation between changes in these polysaccharides and changes in gross viscosity.

The present study is an attempt to establish the role of water insoluble solids (WIS) and soluble solids as contributors to the gross viscosity of tomato juices and tomato juice concentrates. The composition

and structural features of tomato WIS as well as the relative contribution of WIS constituents to gross viscosity is described. The influence of processing factors on the amount and composition of WIS and on gross viscosity is also studied.

# 2. REVIEW OF LITERATURE

## 2.1. The tomato: production, general composition, processing

The tomato forms a part of the genus Lycopersicon, a member of the Solanaceae family. The Lycopersicon genus is normally subdivided into two groups; namely the Eulycopersicon and the Eriopersicon. Fruits of the Eriopersicon, which is native to the Andean region (Chile, Peru, Ecuador), remain green throughout development. The red coloured Eulycopersicon contains two species: Lycopersicon pimpinellifolium, known as the currant tomato, and the cultivated tomato Lycopersicon esculentum (Rick 1978, Sims 1980, Davies & Hobson 1981, Goodenough 1981). The origin and early events of the tomatoe's domestication remain rather obscure, although it is generally believed that Mexico is the region of original domestication. In the Nahua language of Mexico the plant is known as tomatl which is undoubtedly the origin of the modern name. The cultivated form of the tomato probably originated from the wild form L. esculentum var. cerasiforme, known as the cherry tomato. Shortly after the discovery of the New World the tomato was introduced to the European region. The first descriptions on the occurrence of the tomato plant in southern Europe were published by Pier Andrea Mattiolus who, in 1544, described a plant known as pomi d'oro, mala aurea (golden apple) or pomo amoris (love apple).

Because of its known relationship to the nightshades, which also belong to the Solanaceae family, it took until the middle of the 19th century for the tomato to be generally accepted as a food item. Since then, world production of tomatoes for consumption has increased and in 1979 it ranked third in the world production of fruits after the production of grapes and citrus fruits (Davies & Hobson 1981). In general, 70-80% of the total annual tomato crop is used in the tomato processing industry (Rick 1978, Moresi & Liverotti 1982).

Table 1 shows production data for the U.S. and three E.E.C. countries for the years 1982-1985.

Table 1: Production of fresh tomatoes (tonnes) for processing industry.

| | 1982 | 1983 | 1984 | 1985 |
|---|---|---|---|---|
| U.S. total | 7.298.990 | 7.029.840 | 7.679.570 | 7.021.320 |
| California | 6.148.000 | 5.972.930 | 6.591.750 | 6.020.000 |
| Italy | 3.037.900 | 4.348.296 | 5.597.327 | 3.669.973 |
| Greece | 1.008.696 | 1.076.211 | 1.600.000 | 1.300.010 |
| France | 375.367 | 304.500 | 355.000 | 391.233 |

Sources: Annual Vegetables, Crop Reporting Board, SRS, USDA, June 1983.
California Vegetable Review, vol 6 (10), 1985.
Data for Europe were kindly supplied by mr. R. Gandolfi, Parma, Italy.

Although the U.S. Supreme Court in 1893 adjudged the tomato botanically to be a fruit, in common language it is still often considered to be a vegetable. Botanically the tomato is a fleshy berry (Fig. 1) covered by an epidermis (skin) consisting of four to five layers of small sized cells under a thin cuticle.

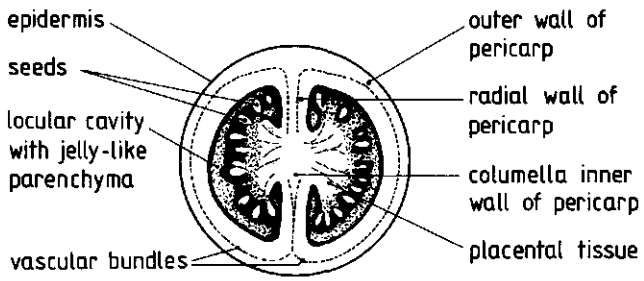



Fig. 1: Transverse section of mature tomato fruit (from: Davies & Hobson 1981).

The body of the fruit is known as the pericarp and consists of outer, radial and inner walls (columella). The locular cavities appear as gaps in the pericarp and contain the seeds embedded in a jelly-like parenchymateous tissue originating from the placenta. The number of locules is two or more,

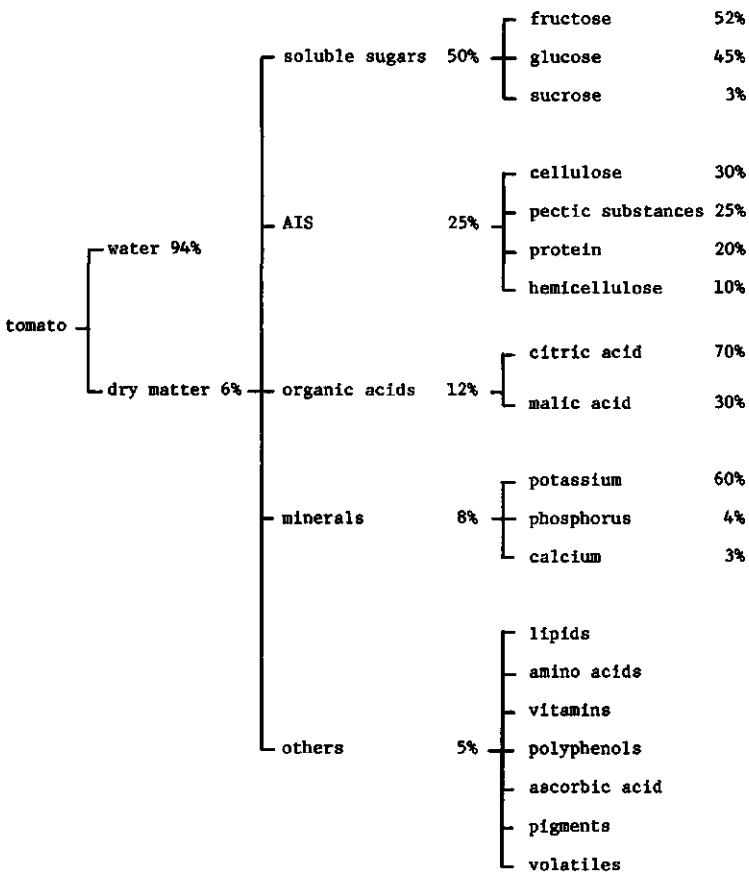
depending on the variety. Vascular bundles radiate from the stem end of the fruit, both round the pericarp and down the columella, to the bottom end. The nutrient value of the tomato is not particularly high. As is shown by Rick (1978) the tomato ranks 16th when compared with other fruits and vegetables on its relative concentration of a group of 10 vitamins and minerals. However, because of the large consumption it stands first in total contribution of these nutrients in the U.S. diet (Table 2).

Table 2: Consumption vs nutritional content of tomatoes in the U.S.

| nutrient concentration | | Contribution of nutrients to diet | |
|---|---|---|---|
| Crop | Rank | Crop | Rank |
| Broccoli | 1 | Tomatoes | 1 |
| Spinach | 2 | Oranges | 2 |
| Brussels sprouts | 3 | Potatoes | 3 |
| Lima beans | 4 | Lettuce | 4 |
| Peas | 5 | Sweet corn | 5 |
| Asparagus | 6 | Bananas | 6 |
| Artichokes | 7 | Carrots | 7 |
| Cauliflower | 8 | Cabbage | 8 |
| Sweet potatoes | 9 | Onions | 9 |
| Carrots | 10 | Sweet potatoes | 10 |
| Sweet corn | 12 | Peas | 15 |
| Potatoes | 14 | Spinach | 18 |
| Cabbage | 15 | Broccoli | 21 |
| Tomatoes | 16 | Lima beans | 23 |
| Bananas | 18 | Asparagus | 25 |
| Lettuce | 26 | Cauliflower | 30 |
| Onions | 31 | Brussels sprouts | 34 |
| Oranges | 33 | Artichokes | 36 |

Table 3 shows the mean composition of tomato fruits. The data are compiled from review articles by Hermann (1979) and Davies & Hobson (1981). Soluble sugars represent approximately 50% of the total tomato solids. Another 25% of the tomato solids is of high molecular weight and insoluble in alcohol (AIS). Other important constituents of the tomato solids are organic acids and minerals. A more detailed composition of this AIS will be discussed in section 2.5.

Table 3: Mean composition of tomato fruit compiled from Hermann (1979) and Davies & Hobson (1981).




As already mentioned, the largest portion of the annual tomato crop is processed. In the EEC countries the main tomato product is tomato paste (Table 4). Italy is, based on quantities, the only producer of passata, a slightly concentrated tomato juice of approximately 8°Bx.

Table 4: Production of tomato products in % of total processed tomatoes (1985).

| | peeled tomato | tomato juice | passata | paste |
|---|---|---|---|---|
| Italy | 28.6 | 3.1 | 8.2 | 59.1 |
| Greece | 1.4 | - | - | 97.6 |
| France | 12.9 | 1.0 | 4.8 | 78.8 |
| total EEC | 20.8 | 2.2 | 5.9 | 69.9 |

Data kindly supplied by mr. R. Gandolfi, Parma, Italy.

Table 5 gives an indication of the amounts of tomato paste produced in some European countries in 1984 and 1985. It shows that Italy is by far the largest producer of tomato paste.

Table 5: Production of tomato paste in Europe (tonnes of tomatoes) in 1984 and 1985.

| | 1984 | 1985 |
|---|---|---|
| Italy | 3.187.894 | 2.169.395 |
| Greece | 1.558.400 | 1.268.890 |
| Turkey* | 930.000 | 900.000 |
| Portugal* | 580.000 | 630.000 |
| Spain* | 480.000 | 350.000 |
| France | 279.000 | 308.267 |

Data kindly supplied by mr. R. Gandolfi, Parma, Italy
* approximate figures.

A simplified scheme for the production of tomato paste is outlined in Fig. 2. More detailed information can be obtained from Nelson & Tressler (1980), Goose (1981), Le Maire (1981) and Gould (1983).

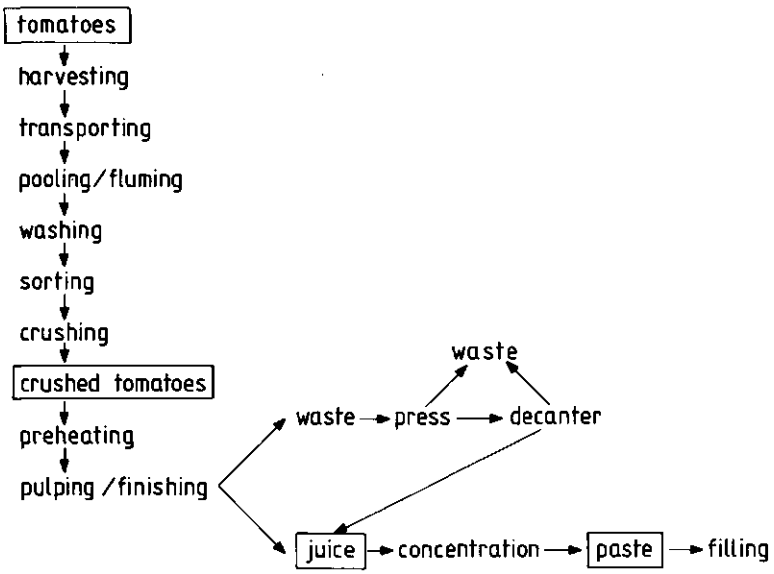



Fig. 2: Flow diagram for the production of tomato paste.
Synonyms found in literature: crushing = chopping = breaking, preheating = scalding, juice = pulp.

After hand picking or mechanical harvesting, the tomatoes are, in general, transported to the processing plant by means of bulk trucks. The trucks are unloaded with large capacity water hoses and flumed to stock pools which provide a continuous supply of tomatoes to the plant. Before being processed the tomatoes are thoroughly washed (5-10 ppm chlorine) and sorted out for defects. The washed tomatoes are chopped into small pieces and heated to a specific temperature. A distinction has been drawn between a hot break process and a cold break process. In the hot break process tomatoes are heated as quickly as possible to a temperature higher than 90°C in order to inactivate pectolytic enzymes, while in the cold break process the tomatoes are heated to any temperature between 40°C and 60°C. Often, the chopping and heating procedure is performed simultaneously in a recirculating chop/scald system consisting of a chopper box and tubular heater. The Rossi & Catelli "Eldorado" chopper/scalder, equipped with a pressurized section, offers the opportunity to heat the tomatoes almost instantaneously to any temperature up to 110-115°C. The hot, crushed

tomatoes pass a two or three stage pulper/finisher unit. Seeds and skins are removed; moreover the juice is refined. Typical values for the pulping screen are 1.0-1.5 mm, while the finisher screens may range from 0.4-1.0 mm depending on tomato quality and product specifications. Many factories increase their yield by pressing the waste material which remains after the pulping/finishing step (Le Maire 1981). The juice obtained after this pressing action is centrifuged to remove impurities, and then the centrifuged juice is added to the refined juice. In the early days of the tomato paste industry the juice was concentrated in open pans; due to the high temperatures the product was very susceptible to caramelisation and scorched flavours. A major improvement was the introduction of vacuum pans or boules; many factories still use these type of evaporators. Modern tomato paste plants, however, use large continuous double or triple effect evaporators, with capacities ranging from 60 to over 250 tonnes of 28-30°Bx tomato paste/24 hours (Goose 1981, Anonymous 1981). The rise in energy costs focussed attention on more energy efficient concentration methods such as the mechanical vapour recompression evaporator and reversed osmosis (Dale et al. 1982). Reversed osmosis is already used on a commercial scale for the preconcentration of tomato juice to approximately 8.5°Bx. The final step in the production of tomato paste is packaging. Traditionally tomato paste has been packed in 5 kg cans. Although large volumes are still packed in this way, more and more tomato paste is packed aseptically in metallized plastic bags (Orbell 1980).

## 2.2. *Definitions and quality parameters for tomato products*

In their standards of identity (Lamb 1977) the U.S. Food and Drug Administration (F.D.A.) defines tomato juice as:

> "the unconcentrated liquid extracted from mature tomatoes of red or reddish varieties with or without scalding followed by draining. In the extraction of such liquid, heat may be applied by any method which does not add water

thereto. Such liquid is strained free of skins, seeds and other coarse or hard substances, but carries finely divided insoluble solids from the flesh of the tomato. Such liquid may be homogenized and may be seasoned with salt."

The name concentrated tomato juice may be used when tomato juice is concentrated to a level not less than 20% natural tomato soluble solids (N.T.S.S., measured on the sugar scale of a refractometer).

Tomato puree and tomato paste are prepared from one or any combination of the following optional tomato ingredients:

1) the liquid obtained from mature tomatoes of red or reddish varieties.
2) the liquid obtained from peelings and cores remaining after preparation of tomatoes for canning, with or without whole tomatoes or pieces of tomatoes.
3) the liquid obtained from the residue from partial extraction of juice from tomatoes.

The method of preparing tomato puree/paste is the same as for tomato juice. As a result of concentration, tomato puree contains not less than 8.0% but less than 24.0% N.T.S.S.; tomato paste contains not less than 24.0% N.T.S.S.

Tomato ketchup is prepared from the same ingredients as tomato puree/paste but in addition salt, vinegar, spices and/or flavourings, onion and/or garlic and sweetening agents (sucrose, dextrose, glucose sirup) are added.

Codex Alimentarius definitions for tomato juice and tomato paste correspond with the F.D.A. definitions for tomato juice and concentrated tomato juice.

Table 6 shows the U.S. Department of Agriculture (U.S.D.A.) standards for tomato juice, puree, paste and ketchup (Lamb 1977).

Table 6: U.S.D.A. standards for tomato juice, puree, paste and ketchup.

| | tomato juice | | | tomato puree/paste | | | tomato ketchup[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| factor | maximum points | grade A | grade C | maximum points | grade A | grade C | maximum points | grade A | grade C |
| colour | 30 | 26-30 | 23-25 | 50 | 45-50 | 40-44 | 25 | 21-25 | 17-20 |
| consistency | 15 | 13-15 | 10-12 | - | - | - | 25 | 22-25 | 18-21 |
| defects | 15 | 13-15 | 10-12 | 50 | 45-50 | 40-44 | 25 | 21-25 | 18-20 |
| flavour | 40 | 33-40 | 27-32 | -[1] | - | - | 25 | 21-25 | 17-20 |
| total score | 100 | 85 | 70 | 100 | 90 | 80 | 100 | 85 | 70 |

1: quality factor, however not rated.

2: grade A ≥33% total soluble solids, grade B ≥29%, grade C ≥25%.

Consistency has reference to the viscosity of the product. The tendency of the insoluble solids to separate, leaving practically clear liquid at the top, is also to be noted in this connection. "Good consistency" in ketchup (score 22-25), for example, means that the product shows not more than a slight separation of free liquid when poured on a flat grading tray; is not excessively stiff and flows not more than 9 cm in 30 seconds at 20°C in the Bostwick consistometer (for description of Bostwick consistometer see: Davis et al. 1954 and section 3.1.).

In general, however, the terms consistency, viscosity and gross viscosity are used interchangeably to describe the viscosity of the whole tomato product; this in contrast to the term serum viscosity which describes the viscosity of the tomato product freed of suspended particles. In this thesis the terms gross viscosity and serum viscosity will be used to describe the viscosity of "whole tomato product" and "serum" respectively.

## 2.3. *Factors contributing to the gross viscosity of tomato products*

### 2.3.1. *Summary*

In view of the extent of section 2.3. the data on the factors contributing to the gross viscosity of tomato products as found in the literature are summarized below. Gross viscosity is an important quality attribute of tomato products, such as juice, concentrates, ketchup and other sauces. In the literature review the factors which contribute to gross viscosity were divided into varietal and horticultural factors, compositional factors and processing factors. It is realized that these factors are interrelated, but for the sake of clarity they were dealt with separately. The influence of temperature and degree of concentration on the gross viscosity of tomato products was not discussed.

The gross viscosity of tomato products depends primarily on the viscosity potential of the tomatoes used for processing. Tomato variety (firmness), climate, degree of ripeness at the time of harvest, damage as result of harvest and transportation, and length of storage before processing, influence the viscosity potential of tomatoes.
In general it has been shown that varieties high in insoluble solids (firm tomato varieties) give products of higher gross viscosity.
Tomato firmness depends not only on the variety but also on the state of ripeness of the tomato fruit. Juices prepared from firm unripe tomatoes are higher in gross viscosity than juices from soft ripe tomatoes. Softening as a result of tomato ripening has always been associated with enzymatic modifications of cell wall polysaccharides. Several authors have observed a decrease in AIS content and cell wall weight and a solubilization of pectin as a result of tomato ripening. The enzyme which has been reported as playing a key role in tomato fruit softening is polygalacturonase.
Ripening has also been found to result in a net loss of galactose and, to a lesser extent, of arabinose from the cell walls; moreover marked changes in the molecular weight of tomato cell wall hemicelluloses have been reported. These changes occurred independently of the pectin solubilization. However the role of enzymes other than PG (β-1,3 glucanase, β-galactosidase, cellulase, pectinesterase) in the softening of tomato fruit has been found

to be doubtful.

The extent to which the fruits are damaged during harvesting, and the time between harvest and processing have been found to be very important for product characteristics. Damage to the tomatoes has been found to result in large serum losses and in reduced serum viscosity. An increased concentration of insoluble solids in the juice, led to increased gross viscosity but negatively influenced other juice characteristics (yield, serum separation).

The effects of other factors which might influence the viscosity potential of tomatoes, like cultural practices (time of planting, method of planting, use of fertilizers) and climate (temperature, amount of sun) are not well documented.

The abundant presence of pectolytic enzyme activity in tomatoes has resulted in the fact that for years the emphasis has been put on the importance of pectin and pectin degradation as factors determining the viscosity of tomato products. Theoretically, however, all the polysaccharide degrading enzymes in tomatoes are able to degrade their substrates and influence product viscosity from the moment the tomatoes are crushed. The tomato has been found to contain various types of polysaccharide degrading enzymes of which the most important are: polygalacturonase, pectinesterase, cellulase degrading enzymes, β-1,3-glucanase and β-galactosidase.

The gross viscosity of tomato products is also influenced by the composition of the products.

A comparison of the data found in the literature has shown disagreement on the importance of the water soluble solids or serum viscosity in contributing to gross viscosity. Many authors have concluded that serum viscosity influences gross viscosity to a great extent. Other authors, on the contrary, have shown that gross viscosity is not affected by large differences in serum viscosity.

The important role of water insoluble solids (WIS) in determining gross viscosity is more generally accepted. Proportion (WIS/TS ratio), size and shape of the water insoluble solids have been reported as influencing gross viscosity.

The alcohol insoluble solids (AIS) have also been found to be highly correlated to gross viscosity. In the available literature, however, there

is no consensus on the relative contribution of the various AIS constituents to gross viscosity. Conflicting results have been found regarding the influence of total pectin, pectic acids, pectinic acids, cellulose and protein on gross viscosity.
Electrolytes such as soluble pectin, citric acid, sodium chloride and calcium chloride, which are present in tomato juice, have sometimes been reported as having a negative influence on gross viscosity.

Apart from being influenced by varietal, horticultural and compositional factors, the gross viscosity of tomato products is also influenced by processing conditions.
The preheating of crushed tomatoes results in the inactivation of enzymes, including the pectolytic enzymes. Heat treatments of 15 sec. at 82°C and 15 sec. at 93°C have been found to inactivate pectinesterase; the inactivation of polygalacturonase was accomplished after 100 sec. at 88°C, 15 sec. at 93°C and 15 sec. at 104°C.
The beneficial effect of higher preheat temperatures on the gross viscosity has often been explained in terms of quicker enzyme inactivation and a better pectin retention. Some authors have concluded that softening of the tissue is also important in determining the effect of preheating on gross viscosity.
A delay between crushing and preheating has usually been found to result in decreased gross viscosity of the finished juice. This negative effect on gross viscosity is determined by the coarseness of the particles in the crushed tomatoes (small particles favour enzyme action), by the temperature and duration of holding, and probably by the variety (amount of pectolytic enzymes). The holding of crushed tomatoes at temperatures in excess of 90°C often resulted in increased gross viscosity of the juice.
The finisher operation, where skins and seeds are separated from the juice, has been reported as influencing both the yield and the gross viscosity of tomato juice. The use of a paddle type finisher resulted in juices higher in gross viscosity than those prepared with a tapered screw finisher. An increase in paddle speed or an enlargement of the finisher screen openings has been found to increase gross viscosity. Almost no data were found on the effect of the finisher temperature on juice gross viscosity. Preheating was, however, found to influence the finisher operation, probably due to the softening of the tomato tissue.

The effects of concentration on the gross viscosity of hot break tomato juice has been studied by only a few authors. Their data have shown that concentration results in a loss in gross viscosity when the concentrate is diluted to the strength of the juice before concentration. The extent of desiccation of the water insoluble solids and their inability to resorb maximally have been proposed as the cause of this phenomenon.
Increases in the gross viscosity of tomato juices and concentrates have been found to occur after homogenization. These increases have been attributed to a decrease in cell size, an increase in total surface area and entanglement of the cell fragments.
The heat processing of tomato juice and paste, before or after packing, has been found to influence gross viscosity negatively. It has been hypothesized that the decrease in gross viscosity was due to a decrease in the molecular weight of pectins.
It has been found that storage does not affect the gross viscosity of tomato juice, but does cause a decrease in the gross viscosity of dilutions of high solids pastes (30°Bx).

### 2.3.2. *Contribution of varietal and horticultural factors to gross viscosity*

The viscosity (gross and serum) of tomato products depends primarily on the viscosity potential of the tomatoes used for processing. Tomato variety, climate, degree of ripeness at the time of harvest, damage as result of harvest and transportation, and length of storage before processing, have been found to influence the viscosity potential of tomatoes.

Pear shaped tomatoes have often been reported to contain more AIS, WIS and total pectin, and to yield juices and pastes higher in viscosity than the round varieties (McColloch et al. 1950, Luh et al. 1954, Smit & Nortje 1958, Twigg 1959, Luh et al. 1960, Cooler 1962, Roy & Choudhury 1972). Bel-Haj (1981) showed that five cultivars which did not differ significantly in chemical composition also failed to show differences in juice gross viscosity. These examples show that varietal differences in composition affect the viscosity of the products. For this reason tomato varieties are often classified according to their viscosity

potential in high-viscosity, medium-viscosity and low-viscosity varieties. In general it has been shown that varieties high in insoluble solids (measured as alcohol or water insoluble solids) will produce products of higher viscosity. In the past 10-15 years numerous newly developed tomato cultivars have been studied with respect to yield and juice characteristics. Based on the results of their studies, Marsh et al. (1982, 1983) state that the most important attribute of a processing tomato cultivar after its soluble solids (NTSS) content, is the % of insoluble solids in the total solids (WIS/TS). These two values provide critical information for planning purposes. NTSS will determine the yield for a product concentrated to some given level of solids, but both NTSS and WIS/TS ratio are necessary to predict the yield for a product which has both gross viscosity and solids specified in its standard of identity. Johanessen (1981), therefore, pointed to the need for developing tomato varieties with high soluble solids and WIS/TS ratios to be used for gross viscosity oriented tomato products. This WIS/TS ratio was found to vary widely in new tomato cultivars (Table 7); however, the ratio increased as a result of the search for tomato varieties suitable for mechanical harvesting (firm varieties).

Table 7: Trends in WIS/TS ratio (from Marsh et al. 1980, 1982, 1983).

| year | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 |
|---|---|---|---|---|---|---|---|
| no. var. | 23 | 12 | 12 | 12 | 12 | 12 | 12 |
| range | 0.101-0.182 | 0.109-0.173 | 0.125-0.185 | 0.137-0.195 | 0.114-0.171 | 0.125-0.186 | 0.130-0.164 |
| mean | 0.135 | 0.139 | 0.152 | 0.167 | 0.145 | 0.166 | 0.152 |

The success in developing firmer tomato varieties, which also yield juices high in gross viscosity is illustrated with the following example. In 1971 the VF 145 variety accounted for about 50% of the mechanically harvested tomatoes in the U.S. This variety was said to have many desirable characteristics highly acceptable to growers as well as processors (Luh et al. 1971). Marsh et al. (1979) considered this variety to be only medium firm and susceptible to damage during mechanical harvest and

transportation. Finally, Luh et al. (1985) classified this variety as a low-viscosity variety.

The development of these firm varieties suitable for mechanical harvesting caused some unexpected problems with the production of cold break tomato paste. Due to the high insoluble solids content of the tomatoes, cold break yields were insufficient and problems were experienced in pumping the paste (Bartholin 1981). To overcome these problems Bartholin (1981) studied the usefulness of adding pectolytic enzymes as well as removing of insoluble solids by centrifuging as a means of decreasing the gross viscosity of cold break tomato paste.

Tomato firmness depends not only on variety but also on the state of ripeness of the tomato fruit. Hall (1963) reported a decrease in AIS content of tomato fruit during ripening. Whittenberger & Nutting (1957) showed that juices prepared from green tomatoes were higher in gross and serum viscosity as well as cell wall and pectin content, than juices prepared from ripe tomatoes. This influence of degree of ripeness on viscosity is confirmed by several other authors (Twigg 1959, Luh et al. 1960, Cooler 1962, Bartolome 1972).

The biochemical changes in fruits accompanying ripening have been extensively reviewed by Pressey (1977), Hobson (1981) and Knee & Bartley (1981). Tomatoes have often been used as a system for studying fruit ripening because of the availability of genetic mutants that do not ripen normally; never ripe (Nr), ripening inhibited (rin), and non-ripening (nor). Softening as a result of tomato ripening always has been associated with enzymatic modifications of cell wall polysaccharides.
Wallner & Bloom (1977) reported a 33% decrease in cell wall weight during ripening from the mature green to the red ripe stage. The enzyme which has been reported as playing a key role in tomato fruit softening is polygalacturonase (PG). PG is absent in mature green fruits but increases dramatically during ripening (Foda 1957, Hobson 1964, Gross & Wallner 1979, Tucker et al. 1980, Tucker & Grierson 1982). Solubilization and degradation of pectin has been reported to coincide with the increase in PG activity (Gross & Wallner 1979, Huber 1983, Rushing & Huber 1984). Hobson (1963)

reported a decrease in total pectin of 37-41% and a loss in insoluble pectin of 87-89%. The ratio insoluble pectin/total pectin decreased. The ripening mutants show decreased PG activity and rate of softening (Nr-mutant), or no PG-activity and softening at all (rin-mutant) (Buescher & Tigchelaar 1975, Tucker et al. 1980, Tucker & Grierson 1982). Hobson (1980) proposed that the very low activities of PG, phosphofructokinase and $NADP^+$-malic enzyme in the fully developed mutant lines offered an explanation for the weak climacteric respiration rise and slow ripening of Nr-fruit and the non-climacteric behaviour of rin-fruit. Firm tomato varieties which ripen slowly also contained less PG activity and showed less pectin degradation during ripening than the softer varieties (Hobson 1965, Tucker et al. 1980, Malis-Arad et al. 1983).

Some years ago it was suggested that PG activity is an essential catalyst for the whole tomato ripening process (Tigchelaar et al. 1978, Poovaiah & Nukaya 1979). However, other authors (Sawamura et al. 1978, Brady et al. 1982, Grierson & Tucker 1983, Crookes & Grierson 1983) clearly showed that PG activity increased not before 2-3 days after the onset of the respiratory climacteric as detected by an increase in ethylene production. It was concluded, therefore, that ripening appears as a coordinated series of events which are set in train by ethylene. Brady et al. (1982) hypothesized that although PG does not have an initiating effect it can play a catalytic role. It is possible that the initial intracellular events, indicated by a rise in ethylene production and respiration, induce PG production. Activity of PG in turn may release catalysts (see Strand et al. 1976) previously inactivated on the walls, which lead to further increases in ethylene and to other intracellular responses of ripening, including e.g. the chloroplast to chromoplast transition. Ethylene production, in turn, may be triggered by the plant hormone auxin (Goodenough 1981). Recently Rushing & Huber (1985) presented results illustrating the role of copper in the biosynthesis and activity of ethylene in ripening fruit.

In the maturing tomato fruit the cells are connected by the middle lamella which is thought to contain large amounts of pectin. The non-esterified carboxylgroups are important in maintaining cohesion by cross-linking with divalent metal ions (calcium). In the cells of mature

green tomatoes a certain degree of cell separation has already occurred as a result of cell elongation during growth (Pressey 1977). Changes in the structure of cell walls as a result of ripening start with the dissolution of the middle lamella; later a more severe disruption of the primary cell wall may occur (Crookes & Grierson 1983). Many authors showed the existence of at least two isoenzymes (Fig. 3) which act sequentially during ripening (Pressey & Avants 1973, Tucker et al. 1980, 1981, Zainon & Brady 1982, Crookes & Grierson 1983).

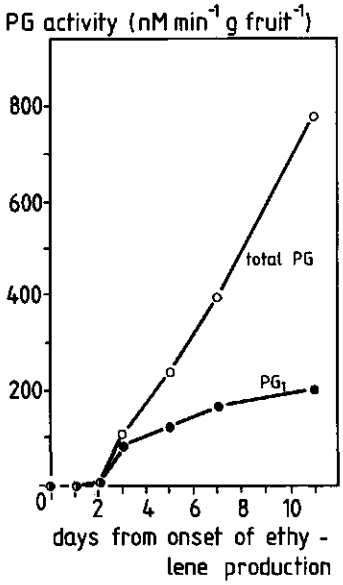



Fig. 3: Changes in PG isoenzymes during ripening (from Crookes & Grierson 1983).

Crookes & Grierson (1983) proposed that $PG_I$ was responsible for the middle lamella dissolution and that the later stage in which the primary cell wall was attacked was due to $PG_{II}$. One to two days after the onset of ethylene production an increase in endoplasmatic reticulum with ribosomes attached to it was detected. It is possible that PG is synthesized on this rough endoplasmatic reticulum just before the appearence of its activity (Crookes & Grierson 1983). Other authors also reported "de novo" synthesis of PG during ripening (Tucker et al. 1980, Tucker & Grierson 1982, Brady et al. 1982).

Chemical analysis of tomato cell walls at several stages of ripening, and the incubation of cell walls from mature green tomatoes with tomato enzyme extracts or purified PG, confirmed the role of PG in softening, but also showed that besides the dissolution of pectin other changes take place in the cell wall. (Wallner & Bloom 1979, Gross & Wallner 1979, Pressey & Avants 1982, Themmen et al. 1982, Crookes & Grierson 1983, Huber 1983, Gross 1984). A net loss of galactose and to a lesser extent of arabinose from the cell walls was found during ripening. Mature green tomato cell walls contain 12-15% galactose while the cell walls of ripe fruits contain only 4-7% galactose. (Wallner & Bloom 1977, Gross & Wallner 1979, Gross 1984). Data presented by Wallner & Bloom (1977) show that the total net loss of galactose in tomato fruit may amount to 60% during ripening. Gross & Sams (1984) showed that other fruits also lost galactose and/or arabinose during ripening. The solubilization of galactose seems to occur independently of pectin solubilization. In vitro addition of tomato enzyme extract to cell walls of green tomatoes failed to solubilize a large part of the galactose in addition to the pectin (Wallner & Bloom 1977). Gross & Wallner (1979) showed that detached rin-mutant fruit lost neutral sugars in absence of PG and pectin solubilization. Both Wallner & Bloom (1977) and Gross & Wallner (1979) indicated that part of the galactose loss already occurred before the onset of ripening. Lackey et al. (1980) suggested that the decline in wall galactan is partly due to reduced synthesis in senescing normal fruits and in detached rin-tomatoes. In attached rin-tomatoes the decline in galactose was much smaller. Indeed Pressey (1983) isolated a β-1,4-galactosidase II which was able to hydrolyse a galactan rich polysaccharide (Pressey & Himmelsbach 1984) into its monomeric constituents. The increase in free galactose that occurs in tomatoes during ripening (Gross & Saltveit 1982, Gross 1983) may be a product of this enzyme. Gross (1984) found that decreases in galactose content occurred in both ionically associated pectin (chelator soluble) and covalently bound pectin (cold alkali soluble) but also in a non-cellulosic polysaccharide fraction which was part of the cellulosic fraction (resistant to extraction with 4 M KOH). Data indicated that at least two galactose containing polymers are involved in the net loss of wall galactose residues during ripening.

In addition to these compositional changes in the cell wall, Huber (1983) observed a marked change in the molecular weight of tomato cell wall hemicelluloses. Hemicelluloses (rich in xylose and glucose) from immature green fruit were similar to those from mature green fruit, whereas hemicelluloses from fruit harvested during ripening showed progressively lower quantities of high molecular weight polymers and higher quantities of low molecular weight polymers (MW <40.000). The changes in hemicelluloses occurred independently of pectin degradation.

Attempts have often been made to detect a role for enzymes other than PG in tomato fruit ripening; this because a certain amount of softening already occurs before the onset of ripening and the increase in PG activity (Hobson 1965, Besford & Hobson 1972). In addition to this, Sawamura et al. (1978) showed that water soluble pectin (WSP) increased before PG was active. They found that an early detachment of tomatoes advanced ripening. The ethylene treshold for an increase in WSP decreased while the treshold for the increase in PG activity did not change. The increase in WSP was attributed to either the activity of an enzyme other than PG, or to "de novo" synthesis of WSP (compare Knee 1982).

The presence of several carbohydrases, of which β-1,3-glucanase and β-galactosidase are the most important, has been reported in tomatoes (Wallner & Walker 1975, Pharr et al. 1976, Hinton & Pressey 1980, Pressey 1983). The role of these enzymes in ripening is still doubtful: on the one hand because these enzymes were not able to initiate wall degradation (Wallner & Bloom 1977) or to degrade a tomato cell wall polysaccharide (Gross & Wallner 1979), on the other hand because it was shown that these enzymes were present throughout growth and ripening (Wallner & Walker 1975, Pharr et al. 1976).

The same is more or less true for the influence of cellulases on softening. Although Hall (1964), Dickinson & McCollum (1964) and Sobotka & Watada (1971) reported a relationship between firmness and cellulase activity, other data do not confirm such a relationship (Hobson 1968); moreover, it has been shown that cellulase is already present in the green fruit (Buescher & Tigchelaar 1975, Wallner & Walker 1979) and will increase also in the rin-mutant (Poovaiah & Nukaya 1979).

Pectinesterase (PE) is not thought to be directly involved in tomato fruit ripening. Hall & Dennison (1960) and Hobson (1963) found no relationship

between PE activity and firmness of tomatoes; other authors showed the presence of PE in green tomato fruits and in fruits of the Nr- and rin-mutants (Buescher & Tigchelaar 1975, Sawamura et al. 1978, Tucker et al. 1982). However, it is proposed that although PE activity does not result directly in softening it may influence cell wall degradation by other enzymes, especially by PG (Tucker et al. 1982). Indeed, Pressey & Avants (1982) showed that purified PG was much more active on deesterified cell walls than on native esterified cell walls (Degree of esterification (DE) ±47%). PE stimulated the action of PG at pH 5, the optimum pH for degradation of deesterified tomato cell walls, and in small amounts at pH 3.5, the optimum pH for degradation of native esterified cell walls. High PE activity at pH 3.5 inhibited cell wall solubilization by PG action. No data were presented on the influence of PE on PG activity at the natural pH of the tomato fruit. However, it was concluded that pectin solubilization during ripening is due to the action of PG on relatively highly esterified substrates. This conclusion is in agreement with the results of Themmen et al. (1982) who showed that cell wall degradation with purified $PG_{II}$ did not differ from cell wall degradation with the total cell wall bound protein extract containing PG, PE as well as β-galactosidase.
Hobson (1981) proposed an inaccessibility of the methoxygroups in protopectin and pectin molecules, preventing PE from quickly and completely deesterifying the substrate, as an explanation for the still high degree of esterification in ripened fruits. If the enzyme could act unrestrictedly on all the pectin in a tomato fruit, the substrate would be completely demethoxylated in four minutes.

Ions also may be important in tomato fruit ripening. Wills & Rigney (1979) found that the activity of PG and PE, isolated from tomato fruits, was inhibited by the addition of high concentrations of calcium ions. According to them, this might explain the complete inhibition of ripening when whole tomato fruits were infiltrated with substantial amounts of calcium. Rigney & Wills (1981) have proposed that the solubilization of cell wall bound calcium is a prerequisite for the initiation of ripening and tissue degradation by PG. Buescher & Hobson (1982) concluded that solubilization of calcium regulates the rate and extent of cell wall degradation by PG during normal tomato fruit ripening. Mizrahi (1980) reported that nor-mutant fruits on plants grown in nutrient solution

containing 3 g/l NaCl exhibited ripening responses which included increases in respiration rate and ethylene production and the development of pectolytic activity and red colour. Rushing & Huber (1984) reported similar effects of calcium and sodium on the solubilization of galacturonic acid from cell walls under the influence of cell bound PG.

The extent to which the fruits are damaged during harvesting (especially with mechanical harvesting) and the time between harvest and processing is very important for both yield and product characteristics (York et al. 1967, Miers et al. 1971, Marsh et al. 1979 b). Damage to the tomatoes during harvest or transportation, and storage of the damaged tomatoes will result in large serum losses and in reduced serum viscosities. As a result of this, the concentration of insoluble solids in the juice may increase giving rise to increased gross viscosity. However, the yield will be very low and serum separation may be negatively influenced by the action of enzymes on serum and insoluble solids.

The effects of other factors which might influence the viscosity potential of tomatoes, like cultural practices (time of planting, method of planting, use of fertilizers) and climate (temperature, amount of sun), are not well documented and will not be discussed here.

### 2.3.3. *Polysaccharide degrading enzymes in tomatoes*

Ripe tomatoes contain several types of polysaccharide degrading enzymes of which the pectin degrading enzymes PE and PG (Fig. 4) are present in very large amounts when compared to other fruits (Hobson 1962, Polacsek-Rácz & Pozsár-Hajnal 1976). Because of this abundant presence of pectolytic enzyme activity, tomatoes have often been used to characterize the nature of plant pectolytic enzymes. On the other hand it has resulted in the fact that for years the emphasis was put on the importance of pectin and pectin degradation as factors determining the viscosity of tomato products. Theoretically, however, all the polysaccharide degrading enzymes in tomatoes are able to degrade their substrates and influence product viscosity from the moment the tomatoes are crushed.

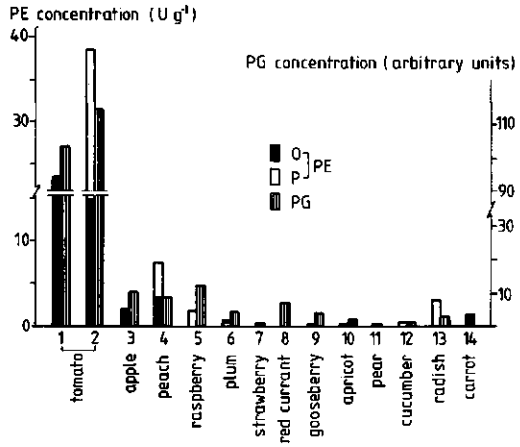



Fig. 4: PG and PE activity in fruits and vegetables
O = PE activity on low-ester pectin,
P = PE activity on high-ester pectin
(from: Polacsek-Ràcz & Pozsar-Hajnal 1976).

The different polysaccharide degrading enzymes will be discussed in the following part.

## *Polygalacturonase (PG)*

In general, polygalacturonases are divided into two groups: exo-PG (EC. 3.2.1.67) removes single galacturonic acid units from the non-reducing end of polygalacturonic acid; and endo-PG (EC.3.2.1.15) hydrolyzes the substrate at random converting it into a mixture of dimer and monomer.

PG activity in tomatoes arises from at least two isoenzymes (Pressey & Avants 1973, Tucker et al. 1980, Zainon & Brady 1982, Moshrefi & Luh 1984) which act sequentially during ripening (Tucker et al. 1980). $PG_I$, which acts at the beginning of the ripening process, is thought to be a dimer of $PG_{II}$ (Tucker et al. 1981). Polyacrylamide gel electrophoresis showed that $PG_{II}$, the dominant PG isoenzyme in ripe tomatoes, consists of two separate polypeptides (Zainon & Brady 1982, Crookes & Grierson 1982, Moshrefi & Luh 1984). The existence of more than one PG isoenzyme was already indicated by McColloch & Kertesz (1948) who found a residual PG activity (20% of initial

activity) after 5 minutes heating in boiling water. Indeed, several authors showed different heat stabilities for $PG_I$ and $PG_{II}$. 50% inactivation of $PG_I$ is obtained after 5 min. heating at 70-78°C while $PG_{II}$ is already inactivated for 50% after 5 min. 52-57°C (Pressey & Avants 1973, Tucker et al. 1980, Zainon & Brady 1982, Moshrefi & Luh 1984). Both isoenzymes are basic glycoproteins (Takehana et al. 1977, Zainon & Brady 1982, Moshrefi & Luh 1984) with a reported pI of 8.6 for $PG_I$ and 9.4 for $PG_{II}$ (Zainon & Brady 1982). The optimum pH for hydrolysis of polygalacturonic acid is approximately 4.5 but this value decreases with a decrease in substrate molecular weight (Patel & Pfaff 1960 a,b, Pressey & Avants 1972, 1973).

Studies with impure tomato PG indicated that during the first 25-30% of hydrolysis, polygalacturonic acid chains are cleaved at random; oligogalacturonic acids were the products of hydrolysis, no free galacturonic acid was found. At 80% hydrolysis mono- and digalacuronic acid were the only reaction products found; the digalacturonic acid was cleaved very slowly. If the initial reaction velocity with polygalacturonic acid is set at 100, those with tetra-, tri-, and digalacturonic acids are approximately 7.0, 1.58 and 1.05, respectively (Luh et al. 1956). Non-purified tomato PG attacked tetra- and trigalacturonic acid from the reducing endgroup (Patel & Pfaff 1960 b). McCready et al. (1955) showed also that monogalacturonic acid was the final reaction product when polygalacturonic acid was incubated with crude tomato PG, indicating some exo-PG activity. However, all the purified isoenzymes of tomato PG are apparently of the endo-type (Pressey & Avants 1973, Markovic & Slezarik 1977, 1981, Takehana et al. 1977, Dongowski et al. 1980, Zainon & Brady 1982). Pressey & Avants (1973) reported that $PG_{II}$ accomplished in 5 min. the same viscosity decrease of a pectic acid solution, with the same increase in reducing endgroups, as $PG_I$ after 30 min. This indicates a different mode of action for the two isoenzymes. The optimal substrate for $PG_{II}$ was found to be a fully demethylated citrus pectic acid. The enzyme still showed ± 20% of the optimum activity on a substrate with a DE of 60% (statistically distributed); no activity was found on a substrate with a DE of 80-90%. $PG_{II}$ showed higher activities on partial esterified polygalacturonic acids (DE <32) with a block-wise distribution of the methylester groups when compared to similar polygalacturonic acids with a

statistical distribution of the ester groups (Dongowski et al. 1980). A positive correlation was found between $PG_{II}$ activity and substrate molecular weight (Dongowski et al. 1980, Markovic & Slezarik 1977, 1981). The mode of action on oligogalacturonic acids was similar to that reported for impure PG (Markovic & Slezarik 1981) with the exception that the final reaction products were mono- and digalacturonic acid; digalacturonic acid was not cleaved.

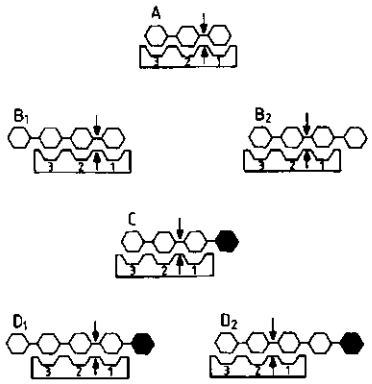



Fig. 5: Enzyme-substrate complex of tomato $PG_{II}$ with oligogalacturonic acids and their reduced derivatives A = trigalacturonic acid, $B_1$ and $B_2$ = tetragalacturonic acid, C = reduced tetragalacturonic acid, $D_1$ and $D_2$ = reduced pentagalacturonic acid, 1-3 = binding subsites, arrow = catalytic site (from: Markovic & Slezarik 1981).

Markovic & Slezarik (1981) concluded that the primary binding site of tomato $PG_{II}$ is composed of three subunits and the catalytic site is situated close to the first bond from the reducing end of the substrate segment bound into the complex (Fig. 5).

*Pectinesterase (PE)*

Pectinesterase (E.C. 3.1.1.11) catalyzes the hydrolysis of methylester groups present in pectinic acids and pectin.

The tomato pectinesterase complex is composed of five to eight multiple forms as is shown by Delincee (1970) and by Rexova-Benkova et al. (1977). Only one type is dominant in ripe tomatoes (Pressey & Avants 1972, Tucker et al. 1982). The enzyme attacks the pectin chain from the reducing

end and at other sites on the substrate molecule, most probably next to a free carboxyl group; the enzyme proceeds linearly along the chain (Lee & MacMillan 1968, 1970). Deesterification results in the formation of segments in the pectin molecule with a block-wise arrangement of free carboxyl groups alternated with segments containing fully esterified galacturonic acid units (Kohn et al. 1983). The partially methylated (60%) pentagalacturonate was found to be the shortest substrate deesterified by tomato pectinesterase (Markovic et al. 1983). The initial reaction rate on the pentamer, however, was only 4.58% of the initial rate on a high molecular weight pectin with a DE of 65%. In general the reaction rate increases with an increase in substrate molecular weight. (Markovic et al. 1983). Polygalacturonic acid and reaction products of oligomeric substrates competitively inhibit the activity of tomato pectinesterase (Lee & MacMillan 1968, Markovic et al. 1983). Markovic et al. (1981), however, found that the purified dominant form of PE was able to deesterify a purified citrus pectin (DE 64.3%) within three hours to a DE of 1.8 ± 0.5% without degradation of the galacturonic acid chains.

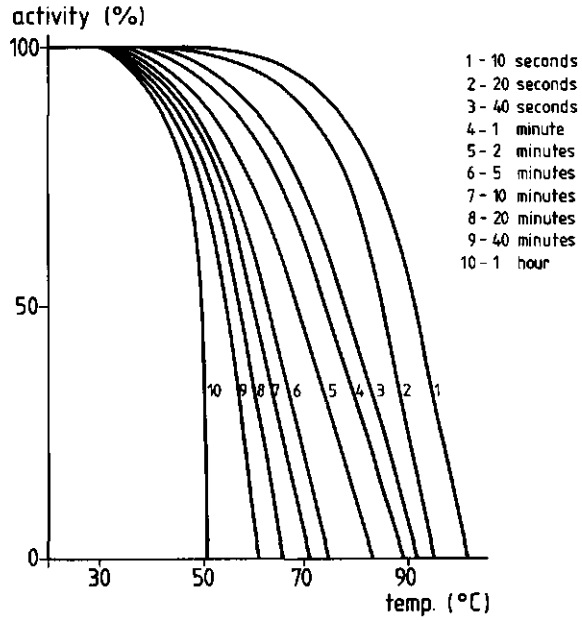



Fig. 6: Thermal inactivation of tomato PE (from: Draetta et al. 1979).

Tomato PE is a basic protein (Delincee & Radola 1970) with a pH optimum in the range 7-8 (Lee & MacMillan 1968, Nakagawa et al. 1970, Pozsar-Hajnal & Polacsek-Rácz 1975, Draetta et al. 1979, Tucker et al. 1982).The enzyme is still active at the natural pH in tomato fruit; however, the activity is dependent upon the DE of the substrate. Pozsar-Hajnal & Polacsek-Rácz (1975) reported lack of PE activity on a 70% DE substrate below pH 5.0; however, on a substrate with a DE of 30-36% tomato PE was still active at pH 4.5 (±30% of activity at optimal pH).

50% of tomato PE activity is lost after 5 min. heating at 60-67°C (Pressey & Avants 1972, Nakagawa et al. 1970, Tucker et al. 1982); data on time and temperature combinations for complete PE inactivation are presented by Draetta et al. (1979) (Fig. 6).

*Cellulases*

The cellulase group of enzymes is classified, according to the mode of action, in endo-glucanase, exo-glucanase and cellobiase.
Endo-glucanase (E.C. 3.2.1.4.), 1,4-β-D-glucan glucanohydrolase, hydrolyses cellulose polymers at random, the minimum substrate size being a trimer. The enzyme is mainly active on amorphous cellulose and on soluble derivatives as carboxymethylcellulose (CMC); it rapidly decreases the viscosity of CMC solutions.
Exo-glucanase (E.C. 3.2.1.91), 1,4-β-D-glucan cellobiohydrolase, attacks from the non-reducing end of the polymer and releases molecules of cellobiose. It is active on crystalline cellulose (Avicel, Whatman cellulose powder) but shows minor activity on CMC; activity decreases with increasing chain length.
Cellobiase (E.C. 3.2.1.21), a β-glucosidase, hydrolyses cellobiose into two glucose molecules.
Selby & Maitland (1967) showed a large synergistic effect when the cellulase group of enzymes produced by Trichoderma viride acted together on cotton fibre. It is probable that endo-glucanase provides the non-reducing end groups necessary for exo-glucanase activity. Cellobiose, which inhibits both endo- and exo-glucanase activity, is degraded by cellobiase.

The presence of the endo-type of glucanase in tomatoes has often been reported (Dickinson & McCollum 1964, Hall & Mullins 1965, Hobson 1968, Sobotka & Watada 1971, Buescher & Tigchelaar 1975, Wallner & Walker 1975,

Poovaiah & Nukaya 1979). Information on the existence of exo-glucanase and cellobiase, however, is rather limited. Pharr & Dickinson (1973) reported activities of endo-glucanase and cellobiase in tomatoes. Cellobiase weakly inhibited endo-glucanase activity. Cellobiase was found to be less heat stable than endo-glucanase. A 50% cellobiase inactivation at 50°C was obtained after 3 min. compared to 40 min. for endo-glucanase. Much of the information on the occurrence of a tomato cellulase-complex is obtained from a study by Sobotka & Stelzig (1974). They detected two fractions with endo-glucanase activity (CMC-liquifying), one with a pH optimum of 7.5 the other with a pH optimum of 5.2. Moreover, these fractions were able to hydrolyse Avicel, Whatman fibrous cellulose powder and bean hypocotyl cell wall material. The fraction with a pH optimum of 7.5 showed properties pointing to the presence of some exo-glucanase activity. This enzyme was active on insoluble celluloses; it degraded short chain cellulodextrins (except cellobiose) and showed CMC-saccharifying activity which was indicated by the release of large amounts of reducing groups.
In addition to the endo-glucanase, a non specific β-glucosidase, active on a number of short chain cellulodextrins and p-nitrophenyl-β-D-glucoside, was isolated. The activity of this enzyme decreased with an increase in chain length. Moreover, an exo-β-1,4-glucanase, highly active on cellobiose, cellotriose, cellotetraose but not on p-nitrophenyl-β-D-glucoside, was found. The total complex of tomato cellulase was able to degrade completely the insoluble cellulose from different sources.

*Other glycanases*

Only a few authors have studied the presence in tomatoes of glycanases other than those belonging to the cellulase complex. One of the glycanases found in tomatoes is β-1,3-glucanase (laminarinase, E.C. 3.2.1.6), an enzyme which hydrolyses the glycosidic bond in a β-1,3 linked glucose polymer. This enzyme, with a MW of 12.000, is present in the same quantities as PG (Wallner & Walker 1975). β-1,3-glucanase has a pH optimum in the range 5-6 and is rather heat stable, as it is inactivated for only 15% after 5 min. heating at 60°C (Hinton & Pressey 1980). Wallner & Walker (1975) found only little or no activities on substrates such as glucomannan, galactomannan (guar gum), β-1,6-glucan (postulan), arabinogalactan (stractan) and xylan.

*Glycosidases*

Usually the presence of glycosidases is tested on p-nitrophenyl glycosides. Using these substrates, activities of α- and β-galactosidase (Wallner & Walker 1975, Pharr et al. 1976, Pressey 1983), α-mannosidase (Pharr et al. 1976) and β-glucosidase (Pharr et al. 1976), were found in tomatoes. The β-glucosidase showed only weak activities on cellobiose and gentobiose (β-1,6 linkage), while the activity on laminarin was somewhat higher but still not more than 10% of the p-nitrophenyl-β-D-glucosidase activity. One of the β-galactosidases isolated by Pressey (1983) was capable of hydrolysing a polysaccharide that was isolated from tomatoes and that consisted primarily of β-1,4 linked galactose. No or only weak activities of α-D-glucosidase, α- and β-L-fucosidase, α- and β-D-xylosidase and β-D-mannosidase were detected in tomatoes (Wallner & Walker 1975, Pharr et al. 1976).

To obtain a tomato product of high viscosity all these enzymes have to be inactivated, either before, during or immediately after crushing, in order to prevent their degradative action on tomato polysaccharides. Tomato enzymes can be inactivated by heat (Kertesz & Loconti 1944) or by addition of enzyme inhibitors (Wagner et al. 1967); the former method, however, is commonly used.

During heat inactivation of the enzymes the temperature always passes through a range where enzyme activity is optimal. Therefore, the heating rate should be high enough to prevent a long residence time of the crushed tomatoes at the optimal temperature for enzyme action. Moreover, the final temperature should be high enough for complete enzyme inactivation. The temperatures recommended for the heat inactivation of enzymes has changed over the last 40 years from 82°C (Kertesz & Loconti 1944) to 85°C (McColloch et al. 1950), 93°C (Twigg 1959) and higher than 93°C (Luh & Daoud 1971). Tomato enzymes can also be inactivated "in situ" by a heat treatment of the whole tomatoes before crushing. This method was found to be preferable to the heating of crushed tomatoes, unless the final temperature of the heated tomatoes was insufficient for complete enzyme inactivation (Kertesz & Loconti 1944, McColloch et al. 1952, Cooler 1962, Crandall & Nelson 1975). Because of the rapid action of pectolytic enzymes, Wagner and Miers (1967) searched for enzyme inhibitors to prevent the

enzyme catalyzed loss in viscosity during preparation of tomato juices. Strong acids were the most effective inhibitors among the compounds tested.

### *2.3.4. Contribution of compositional factors to gross viscosity*

#### *Contribution of water soluble solids (serum viscosity) to gross viscosity*

Kertesz & Loconti (1944) concluded that a viscous serum is an indispensable prerequisite for a tomato juice of desirable gross viscosity. Results of serum exchange experiments and experiments with enzymatically degraded serum viscosity or artificially increased serum viscosity, led to the conclusion that serum viscosity strongly influences gross viscosity. In the serum the presence or absence of pectic constituents was thought to be of great importance. Robinson et al. (1956) suggested that gross viscosity is the result of "particle" viscosity and serum viscosity. Their assumption was based on analogy with colloidal solutions where viscosity is related to the viscosity of the solvent and the volume of the colloid in solution. Cooler (1962) developed a theory of a weak serum gel which was thought to be necessary for high gross viscosity. The formation of the serum gel was dependent upon the preservation of soluble pectins. Other authors also reported high correlation coefficients between the serum viscosity and gross viscosity of tomato pulp or paste samples (Stevens & Paulson 1976, Marsh et al. 1980, Bel-Haj 1981, Luh et al. 1984). Stevens & Paulson (1976) found indications that, in most situations, either serum viscosity or gross viscosity could be used with considerable confidence to evaluate genotypic variation in viscosity. Marsh et al. (1980) showed that both serum viscosity and the WIS/TS ratio made an important contribution to the Bostwick value of a juice. On the basis of a theoretical model they predict the effect of changes in serum viscosity on the gross viscosity of juices and concentrates. Bel-Haj (1981) considered serum viscosity to be an important factor in altering gross viscosity, Luh et al. (1984 a) found a high correlation between gross and serum viscosity and concluded that both pectinic and pectic acid fractions contribute to the gross viscosity of canned juices and pastes.

In contrast to the above cited authors, Hand et al. (1955) found no relationship between serum viscosity and gross viscosity. In juices of uniform gross viscosity and partical size, a 100% difference in serum viscosity had no perceptible effect on gross viscosity as determined by a panel. They showed that serum viscosity was approximately 1% of the gross viscosity of a juice. Similar results were reported by Robinson et al. (1956), Twigg (1959) and Marsh et al. (1979 b). Robinson et al. (1956) found that serum viscosity is proportional to the soluble pectin content and they stated that pectin is the only constituent contributing to serum viscosity. In general it appeared that when the pectin content of the serum was high the amount of pectin associated with the suspended particles was also high. This is probably the reason for a correlation between serum viscosity and gross viscosity. However, Robinson et al. (1956) prove that by adjusting preheating and finishing conditions, juice samples can be prepared with very different gross viscosity/serum viscosity ratio's, indicating that serum viscosity does not determine gross viscosity. This finding is supported by Twigg (1959), who concluded that serum viscosity was completely unsatisfactory as a single predictor of gross viscosity. Very low coefficients of correlation (r=0.29) were reported between ketchup gross viscosity (sensory panel) on the one hand and serum viscosity (Ostwald-Cannon-Fenske pipette) on the other hand. Marsh et al. (1979 b) presented results which indicate that low serum viscosities as a result of enzyme action during crushing did not result in an increased ketchup yield factor. This factor was defined as the percentage of tomato solids required to make a standard batch of ketchup having a Bostwick value of 6 cm at 33% total solids.

Whittenberger & Nutting (1958) even pointed to the negative effect of the serum on gross viscosity. According to them the serum contains substances (electrolytes) which inhibit the development of maximum gross viscosity in whole juice.

Becker et al. (1972) reported that individually, neither the serum soluble polymers nor the cell wall fibers can cause gross viscosity changes. In the presence of intact cells substantial increases in the serum viscosity resulted in minor gross viscosity increases. Increased amounts of dispersed (=homogenized) cell wall fibers also resulted in minor viscosity increases, unless a high viscosity serum was present. When a

juice contained both large amounts of dispersed cell wall fibers and a high viscosity serum, the gross viscosity was greatly elevated. These results may indicate that the influence of serum viscosity on gross viscosity depends upon the physical state of the cell walls.

### *Contribution of water insoluble solids (WIS) or suspended particles to gross viscosity*

Many authors report on the influence of water insoluble solids or suspended particles on the gross viscosity of tomato products. The majority of them agree with the conclusion of Kertesz & Loconti (1944) that the proportion of insoluble solids, as well as their shape, size and other properties, are of importance in determining the gross viscosity of tomato juice. Whittenberger & Nutting (1957, 1958) dissected tomatoes into four fractions: shell tissue 48%, placenta or center tissue 26%, free juice occurring in the vicinity of the seeds 18%, and gelatinous envelopes surrounding the seeds 8% (Fig. 7).

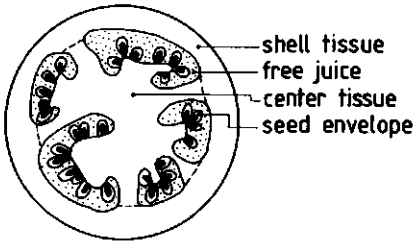



Fig. 7: Cross-section of tomato fruit (from: Whittenberger & Nutting 1957).

Juices from the outer shell and from the center tissues were moderately thick and contained moderate quantities of cell walls. Juice from the gelatinous seed envelopes was thick and contained cell walls heavily impregnated with pectin. Free juice from the seed cavities was exceedingly thin and was devoid of cell walls. They concluded that the cell wall, comprising only 6% of the total solids and less than half of the insoluble solids, was the structure most closely related to the gross viscosity of tomato juice.

York et al. (1967) found a high correlation between the amount of insoluble solids and the Bostwick consistency of tomato products. Bartolome (1972) also concluded that the amount of insoluble solids had the greatest influence on juice gross viscosity, but that the effects of serum viscosity, titratable acidity and the nature of the suspended particles were also important. Foda & McCollum (1970) reported a great reduction in gross viscosity when washed solids were resuspended in their original serum. They concluded that high molecular weight polymers with a high ratio saccharide/uronide and associated with the insoluble solids may play an important role in determining gross viscosity.

Marsh et al. (1980) studied the influence of composition on the rate of change in gross viscosity during concentration. They found that the change in Bostwick consistency of tomato juice as result of a change in solids content during concentration was related to the WIS/TS ratio and to the viscosity of the serum of clarified juice. These characteristics were found to be widely variable among new tomato cultivars. The slope of the concentration curve (log Bostwick = A % solids + B, Luh 1954) was primarily dependent upon the WIS/TS ratio; the locus of the concentration curve, on the other hand, was found to be dependent on both WIS/TS ratio and serum viscosity and could be located by the Bostwick consistency of the juice and initial solids content. WIS/TS ratio is a function of cultivar, heat treatment and finisher screensize, while the serum viscosity depends upon the effectiveness of the heat treatment in destroying the enzyme systems. Marsh (1977) reported that for 67 samples the relationship between WIS/TS ratio and Bostwick value at 12° Bx had a correlation coefficient of -0.95. Luh et al. (1984 b,c) also considered the WIS/TS ratio indicative for pulp and paste viscosity.

Marsh et al. (1979 a,b) tried to study and determine the factors responsible for the quality and gross viscosity of ketchup. For this purpose they developed a method of determining the percentage of tomato solids required to make a standard batch of ketchup having a Bostwick value of 6 cm at 33% total solids. The amount of tomato solids expressed as % of the total solids was defined as the yield factor. Gross viscosity and serum separation were found to be unrelated quality attributes. Gross viscosity was found to be directly dependent upon the fraction of water insoluble solids of the total solids of the tomato juice used. If ketchup was prepared from low solids pastes which require almost no dilution, a good

correlation was found between WIS/TS ratio of the juice before concentration and the yield factor ($r = -0.97$). Multiplying ratio and yield factor yielded a constant factor K of 5.38 ± 0.12 (for 24 different varieties). In fact, this factor indicates that all ketchups with 33% solids and a Bostwick of 6 cm contained the same WIS content. This constant also shows that the yield factor can be predicted when the WIS/TS ratio of the juice is known. Marsh et al. (1982, 1983, 1984) also studied the usefulness of the WIS/TS ratio of juice as a reliable predictor for the gross viscosity potential of a high solids paste. It was found that the WIS/TS ratio of juice does not predict the gross viscosity potential of high solids pastes as accurately as the gross viscosity potential of a low solids paste. It is probable that concentration, storage and subsequent dilution altered the physical properties of the carbohydrate polymers. Marsh et al. (1983) presented results which show that a standard batch of ketchup prepared from a high solids paste (24-33%TS) needed approximately 9% more tomato solids than a batch prepared from a low solids paste (13-17%TS). Marsh et al. (1977) reported that the more water was required to dilute a concentrated sample to 12° Bx the higher was the Bostwick value reported for the product and the further it deviated from the value before concentration. The extent of dessication of the water insoluble solids and their inability to resorb maximally is proposed as the cause of the phenomenon.

Smit & Nortje (1958) found that the holding of crushed tomatoes at 21-27°C before preheating resulted in higher gross viscosities of juice, puree and paste. As the WIS content was rather similar for samples with and without holding, it was suggested that the difference in gross viscosity was due to a qualitative (structural) difference in insoluble solids.

Hand et al. (1955), studying the effects of process conditions on gross viscosity, concluded that the quantity of cellular material alone was not the only factor governing gross viscosity; the configuration or shape of the cell fragments was also important. During juice manufacture the cells may become separated, distorted and broken, although many of the structural parts are still recognizable in the finished juice. They agree with Whittenberger & Nutting (1957) that, in general, sheetlike or rodlike walls or wall fragments offer more resistence to flow and give a more stable juice than spherical cells. The irregularity of cell wall form

depends largely on the mechanical treatment the walls receive during juice manufacture. Forcing cell walls through passages of small clearance, and other types of shearing, stirring or beating actions increase linearity (Whittenberger & Nutting 1957). Kertesz & Loconti (1944) reported that the particles suspended in tomato juices were of irregular shape and to a certain extent resembled cogwheels. These irregularities were explained by a number of factors such as tearing of the tissue during manufacture and swelling or uneven corrosion during heating. Becker et al. (1972) studied the effect of pH during breaking on the gross viscosity of tomato juice. Juices prepared at the natural pH had almost no broken cells; juices prepared at low pH had very high gross viscosities but also high amounts of broken cells.

In contrast to all these authors McColloch et al. (1950) found no relationship between insoluble solids content and paste gross or serum viscosity as measured with the penetrometer and the rate of filtration test.

Twigg (1959) calculated that 49% of the variations in ketchup gross viscosity as determined by a panel could be attributed to the compositional factors, structural solids, non structural solids, total pectin and pH. The relative influence of the structural solids (= total AIS-total pectin) was only 14%.

### *Contribution of AIS to gross viscosity*

The importance of AIS as a contributor to the gross viscosity of tomato products is well recognized. However, AIS is mainly used either for the calculation of other components or for the extraction and determination of total pectin and pectin fractions. Twigg (1959) used the AIS contents of ketchups to calculate the structural solids (=AIS-total pectin) and non-structural solids (= total solids-AIS). Cooler (1962) calculated the particulate-AIS fraction by substracting the serum-AIS from the total-AIS. He observed increases in all AIS fractions and increases in the gross viscosity of tomato juice as a result of changes in processing conditions. McColloch et al. (1950) and Luh et al. (1954 a,b) found good correlations between AIS content on the one hand and the gross viscosity of tomato paste

prepared from San Marzano and Pearson tomatoes on the other hand. Both authors pointed to the fact that constituents in the AIS other than pectin were important contributors to gross viscosity. Luh et al. (1954 a,b) showed that paste with a pectin content of 1.53% (dry basis) prepared from San Marzano tomatoes by a cold break process had a higher gross viscosity (Bostwick at 12°Bx) than paste with 3.48% pectin prepared from Pearson tomatoes by the hot break process. Based on an AIS composition of 10% pectic substances, 30% crude fiber, 5% ash and 20-50% nitrogen as protein, McColloch et al. (1950) proposed that protein might be an important component contributing to gross viscosity. Wagner et al. (1969) reported increased gross viscosity and AIS content of juices as a result of the addition of acid during the hot break. They ascribed these effects to quicker enzyme inactivation and better extraction and dispersion of constituents.

Alcohol insoluble solids content of tomato fruits and the gross viscosity of juice were found to be highly correlated (Janoria & Rhodes 1974). The correlation coefficient values, based on 12 cultivars were: $r = 0.97$ for AIS of whole fruit extracted with 50% ethanol and $r = 0.94$ for extraction with 75% ethanol. The correlation of AIS with gross viscosity was high for outer pericarp, $r = 0.93$ and inner pericarp, $r = 0.78$ but low for locular contents, $r = 0.18$. Janoria & Rhodes (1974) conclude that a cause-and-effect relationship exists between a certain fraction of AIS and gross viscosity.

Stevens and Paulson (1976) used genotypic variation in composition and viscosity to study the effects of the various components of the AIS on viscosity. They state that any attempt to isolate a component of tomato juice and to determine the relative effect of this component on juice gross viscosity will destroy interrelationships between components and in this way undoubtedly affect the gross viscosity expression of these components. After preparing AIS from the different tomato puree samples they fractionated the AIS according to the reproduced scheme (Fig. 8). Amounts of polysaccharides (PS) and polygalacturonides (PG) were determined colourimetrically with anthron and carbazole respectively. Total AIS was found to correlate very well with gross viscosity ($r = 0.94$). Stepwise regression analysis of the data showed that water soluble and water insoluble polygalacturonides accounted for most of the variation in gross

viscosity (WSPG, WISPG). The data indicated that water insoluble, pectinol solubilized polysaccharides (WISPS) have the potential for making a large contribution to gross viscosity at higher concentrations. The water soluble polysaccharides (WSPS) and acid hydrolysed polysaccharides (AHPS) contributed little to gross viscosity.

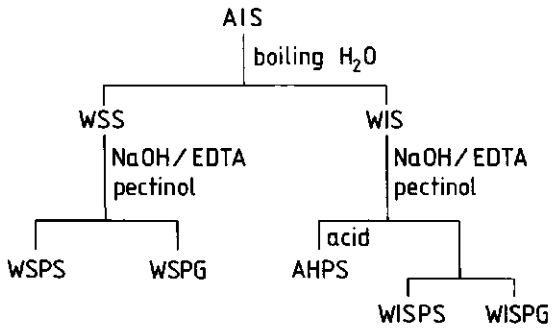



Fig. 8: Scheme for fractionation of AIS (from: Stevens & Paulson 1976).

*Contribution of pectin to gross viscosity*

Most of the information on the contribution of pectin to the gross viscosity of tomato products is obtained from studies related to the inactivation of pectic enzymes by heat (Kertesz & Loconti 1944, Robinson et al. 1945, McColloch et al. 1950, Luh et al. 1954, Luh & Daoud 1971, Garces & Luh 1972, Sherkat & Luh 1976, Sherkat & Luh 1977, Bel-Haj 1981, Luh et al. 1981, 1982, 1984 a, 1984 b, 1984 c) or by acid (Wagner et al. 1967, Miers et al. 1967, Becker et al. 1968, Wagner et al. 1968, Wagner et al. 1969). The use of high hot break temperatures results in quick inactivation of the pectolytic enzymes, a high retention of both total as well as serum pectins and a high gross viscosity of juice, puree or paste. According to Kertesz & Loconti (1944) the soluble pectin will determine the serum viscosity while the insoluble pectic substances will determine the properties (e.g. separation) and the contribution to gross viscosity of the suspended particles. Failure to inactivate pectolytic enzymes early in the processing procedure results in a rapid destruction of the pectic substances in the crushed tomatoes, which in turn results in pastes of low

pectin content and low gross viscosity (McColloch et al. 1950). The addition of acid during the hot break influences juice gross viscosity by at least 2 mechanisms: a) juice gross viscosity and pectin content increase as a result of retarded or prevented enzyme action and b) juice gross viscosity and pectin content increase as a result of increased extraction or improved dispersion of tomato components (Wagner et al. 1969).

Twigg (1959) studied the factors contributing to the gross viscosity of ketchup. Multiple regression analysis with the four variables: structural solids, non structural solids, total pectin and pH, yielded a correlation coefficient of 0.70. This meant that approximately half of the variation in ketchup gross viscosity as detected by a sensory panel could be attributed to these four factors. The relative influence of the factor total pectin on the explainable gross viscosity variations was found to be 44%. Luh et al. (1954, 1981, 1982, 1984 a, 1984 b, 1984 c) reported that tomato juices and diluted tomato pastes with the highest total pectin content also had the highest gross viscosities as measured with the Bostwick consistometer, Stormer viscosimeter or Libby pipette. Luh et al. (1954) found that Stormer viscosimeter readings of commercial diluted paste samples at 10°Bx soluble solids correlated well with the pectin content on a dry weight basis ($r = 0.88$). On semi-logaritmic basis the coefficient of correlation increased to 0.97.

The pectic substances represent a complex mixture of polysaccharides which can be partially extracted from the cell walls with water (pectinic acids with a high degree of esterification) or with aqueous solutions of chelating agents such as EDTA, sodium oxalate or sodium hexametaphosphate (pectic acids with a low degree of esterification). Approximately 60-80% of the total pectic substances present in the cell walls of parenchymatous tissues are extractable. The insoluble pectic material is always found in close association with other cell wall constituents, particularly the -cellulose fraction (Luh et al. 1984 a). Both pectinic and pectic acid fractions of tomatoes contribute to the gross viscosity of canned juice and paste. Higher gross viscosity is not only explained by the larger quantity of pectin retained after processing but also by the weight-average molecular weight of the pectic fractions. Larger carbohydrate polymers are

capable of holding more water, resulting in higher gross viscosity (Luh et al. 1984 a, c).

Whittenberger & Nutting (1957) and Smit & Nortje (1958) detected significant decreases in gross and serum viscosities when tomato juice or diluted paste was treated with a commercial pectinase. This decrease was not accomplished by a clear change in the microscopic appearence of cell walls although the walls appeared to increase slightly in birefringence (Whittenberger & Nutting 1957). The character of cell walls was found to vary with their pectin content. Walls permeated with pectins are tacky, resilient, and capable of binding appreciable quantities of water, whereas walls devoid of pectins are brittle, friable and less hydrophilic. Under identical conditions the pectin containing walls yielded the thicker juices (Whittenberger & Nutting 1957). Whittenberger & Nutting (1958) presented results which deemphasize the role of soluble pectins in determining gross viscosity. As an electrolyte it even had a negative effect on the gross viscosity of tomato juice. On the other hand Cooler (1962) postulated that juices from effectively steam blanched tomatoes existed as a weak gel due to the presence of a continous structure of hydrophylic, macromolecular polyelectrolytes (mostly pectins). Cell walls and other suspended particles were thought to thicken the system and to increase the shearing force necessary to induce and maintain its flow. Stevens & Paulson (1976) emphasized the importance of both insoluble and soluble polygalacturonides in determining gross viscosity. ± 90% of the differences in the gross viscosity of juices prepared from low and high viscosity varieties could be attributed to these two fractions. It was found that both fractions could be equally important.

Luh et al. (1960) studied the relationship between gross viscosity and insoluble pectin. Washed hot break tomato juice was extracted with 0.5% EDTA at neutral pH. After removal of the EDTA the serum was added back to the EDTA treated juice fraction. It was shown that the tomato juice of unripe tomatoes which contained large amounts of insoluble pectin had a high initial gross viscosity but lost 38% of this gross viscosity upon extraction with EDTA. In soft, ripe tomatoes a large part of the insoluble pectin has been converted to soluble pectin. The juices of soft, ripe tomatoes were lower in gross viscosity, but retained most of this viscosity

(89%) after extraction with EDTA. These results seem to indicate that the EDTA soluble fraction of the juice is an important factor determining gross viscosity. Foda & McCollum (1970) repeated these experiments and found a viscosity drop of 80% after resuspension of the EDTA treated cells in the original serum. Relatively low amounts of uronides and reducing sugars were detected in the EDTA extract; therefore the effect of water extraction on gross viscosity was studied. Readings for apparent viscosity (measured with the Brookfield) were 32 for the original juice, 43 for washed solids and 8 for washed solids in the original serum. High molecular weight polymers with a neutral sugar/uronide ratio of approximately 11, closely associated with the insoluble solids, were extracted during the water washings and were responsible for the gross viscosity drop. Foda & McCollum also showed that degradation of washed tomato cells with pectinase after degradation with cellulase (8 M urea added to inhibit pectolytic enzyme activity) did not result in a further gross viscosity loss. It was concluded that the data did not indicate a significant contribution of the uronides to the gross viscosity of tomato juice.

Stephens et al. (1970), studying the pectin content of tomatoes and juices of four varieties, concluded that the content of pectic substances alone did not determine the gross viscosity of canned juice. They refer to the work of Luh et al. (1954 b) who concluded that not only the quantity but also the quality of the pectic materials was important in forecasting the gross viscosity of tomato products.

### *Contribution of cellulose to gross viscosity*

There are not many reports on the contribution of cellulose to the gross viscosity of tomato products. Whittenberger and Nutting (1957) observed that the addition of a technical cellulase preparation (contaminated with pectinase) to hot break juice resulted in the digestion of pectin (serum viscosity drop) and the bulk of the cellulose as indicated by microscopic examination. The gross viscosity of the juice decreased dramatically, to a level lower than after the addition of pectinase alone. Their results indicate the importance of cellulosic structures in maintaining gross viscosity. Boiling cell walls successively in 2% sulphuric acid and 2% sodium hydroxide and then extracting them with

ethylalcohol and ether, removed all non-cellulosic substances from the cell walls. The treated cell walls were brought back to the original volume with distilled water. The gross viscosity of juice decreased with the treatments, indicating that non-cellulosic components of the walls made an important contribution to gross viscosity. However, the pure "cellulose-walls" yielded suspensions of appreciable gross viscosity (Whittenberger & Nutting 1958). In contrast to the opinion of Whittenberger and Nutting, Twigg (1959) reported that the structural solids, which he considered to be identical to the cellulosic cell walls, exerted less influence on the gross viscosity of ketchup than total pectin. Foda & McCollum (1970) on the other hand attribute an important role to cellulose in determining gross viscosity. A technical cellulase preparation in which pectolytic enzyme activities were inactivated by a treatment with 8 M urea, greatly decreased the viscosity of washed solids. Experiments with a cellulase extracted from tomatoes also decreased the gross viscosity for tomato juice. Their data did not indicate an important role of pectin in determining gross viscosity.

Luh et al. (1960) found that the amount of cellulose did not change substantially during ripening or within season. Therefore they concluded that a relationship between gross viscosity and cellulose did not hold. Luh et al. (1981, 1982, 1984 a), studying carbohydrate polymers in processing tomatoes, showed that tomato paste samples high in total pectin content and with a high average molecular weight of the pectin were high in gross viscosities. Their results also prove that the same paste samples were high in acid detergent fibre (ADF) and cellulose content. Roughly, the ADF was composed of 80% cellulose, and 20% lignin and cutin. Luh et al. (1984 b,c) report that total pectin, ADF, cellulose and WIS/TS ratio are indicative of the gross viscosity value of canned tomato juice and paste.

### *Contribution of protein to gross viscosity*

In spite of the fact that proteins are present in large amounts in the AIS of tomatoes and tomato products (Table 8), the role of protein in determining viscosity is not very well documented.

Table 8: % protein in AIS of tomatoes and tomato products.

| reference | % protein* |
|---|---|
| McColloch et al. 1950 | 20-50 |
| Williams & Bevenue 1954 | 17 |
| Brown & Stein 1977 | 23-29 |
| Davies & Hobson 1981 | ±30 |
| Takada & Nelson 1983 | 20-23 |

* total N expressed as protein

McColloch et al. (1950) found losses in AIS as a result of processing which could not be explained by losses in pectin alone. Based on the amounts present they suggested that protein might play a role in determining gross viscosity of tomato paste. On the other hand Foda and McCollum (1970) showed that the degradation of protein in tomato juice with pronase, which contained no pectinase or cellulase activity, caused only a relatively small loss in gross viscosity. Recently Takada & Nelson (1983) reported that the viscosity of a tomato product model system consisting of citrus pectin and bovine serum albumin, changed as a result of a pH change. They attributed this change to the reversible electrostatic complex formation of pectin and protein but not to weak hydrogen bonds between them. A similar pattern of response of gross viscosity to pH change was found in tomato puree (10°Bx) and in tomato juice (5.5°Bx) prepared by dilution from the puree. The maximum gross viscosity was obtained at pH 4.4, and it was found that the pH-effect was reversible. Tomato puree diluted from paste with a higher solids content (20°Bx) did not show a change in gross viscosity with a change in pH. It was concluded that severe or prolonged heating may denature the protein and stabilize its complex with pectin, resulting in an irreversible complex formation. Takada & Nelson speculated that the pectins associated with the cellulose fibrils in the cell walls make tight complexes or fascicles with proteins, resulting in a strong interaction or network around the cell surface. In this way proteins may contribute to the gross viscosity of tomato products.

*Contribution of electrolytes to gross viscosity*

The gross viscosity of tomato juice is greatly elevated by a complete replacement of the serum with distilled water (Whittenberger & Nutting 1958, Foda & McCollum (1970). The addition of non-electrolytes such as sucrose, glycerin and ethylalcohol to such a washed juice had no effect on gross viscosity. However, addition of small amounts of electrolytes including soluble pectin, citric acid, sodium chloride and calcium chloride resulted in decreased gross viscosity. (Whittenberger & Nutting 1958). According to Whittenberger and Nutting, the surfaces of the cell walls bear electric charges (e.g. insoluble pectin) which help maintain the walls in suspension. In the absence of soluble electrolytes, the charges exhibit their maximum effect. The walls swell, bind water and give rise to increased gross viscosity; in fact, sometimes the juice thickens to a semi gel. In the presence of electrolytes, however, the charges on the walls are neutralized, the walls shrink, and a drop in gross viscosity ensues. The quantity of electrolytes occurring naturally in fresh tomatoes is sufficient to keep gross viscosity at a low level. (Whittenberger & Nutting 1958). Foda & McCollum (1970) concluded that the drastic reduction found in gross viscosity after addition of EDTA (0.5%, neutral pH) to washed tomato juice, and the subsequent increase when it was removed, indicated an ionic role, and not a depectinization as suggested by Luh et al. (1960). The low content of pectin in the EDTA extracts offered additional support for this view.

*pH and gross viscosity*

Twigg (1959) reported a relationship between the pH and the gross viscosity of ketchup as measured with a sensory panel. He postulated that the increase in ketchup gross viscosity with a decrease in pH resulted from increases in the viscosity of the low ester pectic substances dissolved in the ketchup. Data presented in his study indicate that approximately half of the water soluble pectin was sufficiently low in methoxyl content to form calcium pectates.

*Callose and gross viscosity*

Callose, an amorphous polymer of glucose residues in β-1,3 linkages, is widespread throughout the plant kingdom and is formed in certain types of cells in response to injury, ultrasound and high temperature. Callose is insoluble in water, ethanol, hot acid, or hot alkali. It has, however, a strong affinity for water (Dekazos 1972).
Dekazos (1972) suggested that perhaps callose is the plant polysaccharide that is the major contributor to the gross viscosity of tomato products and to texture in tomato fruit. Callose was detected in heated unbruised tomatoes (50°C), artificially bruised tomatoes (35-45°C), mechanically harvested tomatoes and in tomato juice from commercially harvested tomatoes. Callose containing tomatoes were noticeably firmer to the touch. The properties of callose were compared to the properties of the cellulosic cell walls described by Whittenberger and Nutting (1958).

## *2.3.5. Contribution of processing factors to gross viscosity*

*Effect of preheating on gross viscosity*

In the past 40 years many authors have reported on the effects of preheating temperature on the gross viscosity of juice, paste and sauces (Kertesz & Loconti 1944, Robinson et al. 1945, McColloch et al. 1950, Luh et al. 1954, Hand et al. 1955, Whittenberger & Nutting 1957, Twigg 1959, Cooler 1962, Luh & Daoud 1971, Garces & Luh 1972, Sherkat & Luh 1976, 1977, Bel-Haj 1981, Luh et al. 1981, 1982, 1984 a,b,c, Xu et al. 1986). This preheating temperature is defined as the temperature to which tomatoes are heated during the crushing process. Kertesz & Loconti (1944) were among the first to study the causes for the increase in juice gross viscosity as a result of higher preheat temperatures. They stated that preheating tomatoes to 82°C was necessary in order to inactivate PE (they did not detect PG activity in tomatoes) and to obtain a high serum viscosity, and as a result of that a juice of pleasing gross viscosity. Like Kertesz & Loconti, many other authors conclude that the beneficial effect of preheating is due to pectolytic enzyme inactivation and therefore to better pectin retention.

However, the literature shows some disagreement about the degree of the preheat temperature necessary for effective inactivation of pectolytic enzymes. McColloch et al. (1950) showed that in tomato paste production 82°C might not be enough to inactivate enzymes and they recommend a preheat temperature of 85°C. Luh & Daoud (1971) and Garces & Luh (1972) reported that PE was inactivated after 15 sec. 82°C and PG after 15 sec. 104°C. Bel-Haj (1981) indicated that 15 sec. 93°C would be enough for PE and PG inactivation. Luh et al. (1981, 1982, 1984 a) stated that a hot break treatment at 88°C for 100 sec. appeared to be enough for PG inactivation.

Information on the effects of specific preheat temperatures on gross viscosity is abundant but not very consistent. Robinson et al. (1945) detected increased gross and serum viscosities when the preheating temperature was elevated from 69°C to 77°C and then to 93°C. In another series of experiments no difference in viscosity (serum and gross) was found between juices without a preheat treatment (temp. ± 27°C) and those preheated to 51°C. Twigg (1959) who studied the effects of preheating at 66, 79, 93 and 100°C on ketchup viscosity, reported that an increase in preheat temperature above 93°C did not increase gross viscosity. In general, higher temperatures resulted in less serum separation, higher serum viscosity, more total and water soluble pectin, and more AIS and structural solids. However, the intrinsic viscosity of water soluble and oxalate soluble pectin decreased strongly during preheating at 100°C in comparison to 93°C. Cooler (1962) reported that the highest juice viscosities were obtained after steam blanching whole tomatoes (in situ inactivation of enzymes) and heating the coarse chopped tomatoes to 90°C or 120°C. Chopping at room temperature (± 30°C) yielded juices with much lower viscosities, independent of the finishing temperature. Juices with higher viscosities had higher AIS, serum-AIS and particulate-AIS content. Luh & Daoud (1971) and Garces & Luh (1972) studied the effects of preheat temperature and holding time on enzyme inactivation and gross viscosity. Serum viscosity increased when preheat temperatures increased from 71°C to 115°C. Only slight gross viscosity increases were detected as result of an increase in preheat temperatures from 60°C to 82°C. However, when the temperature was increased from 93°C to 115°C, gross viscosity, AIS and total pectin increased substantially. Sherkat & Luh (1976) reported that juices preheated at 100°C or 104°C were significantly higher in gross viscosity than those preheated

at 64°C or 77°C. Bel-Haj (1981) detected no differences between preheating at 66°C or 79°C; however, a preheat temperature of 93°C resulted in significantly higher gross viscosity. Serum viscosity increased with all the break temperatures. Luh et al. (1981, 1982, 1984 a,b,c) studied the effects of preheat temperature on the characteristics of juices from several varieties. Increases in preheat temperature (66, 77, 88, 96 and 107°C) always resulted in higher gross and serum viscosities. Not only did total pectin increase as a result of higher temperatures, but so did acid detergent fibre, cellulose and acid detergent lignin. Cellulose content (wet basis) doubled while total pectin content trebled as a result of the increase in temperature from 66°C-107°C. (Luh et al. 1984 b). All these results were explained in terms of quicker enzyme inactivation (also cellulases: Luh 1984 b) and better retention of components as a result of the higher preheat temperature. However, using this theory it is difficult to explain the beneficial effect of preheat temperatures higher than those needed for an efficient enzyme inactivation.

Xu et al. (1986) studied the rheological properties and microstructure of canned juices and concentrates made from four tomato cultivars at three preheating temperatures (85°C, 96°C and 107°C). The higher gross viscosity of juice and pastes as result of a higher preheating temperature was not only attributed to a higher degree of inactivation of PG and PE but also to a highly disrupted cell structure in the samples.

Hand et al. (1955) observed that, in general, an elevation of the preheat temperature increased the gross viscosity of tomato juice; however, the effects were dependent on the finishing procedure. The effect of preheating temperature on serum viscosity was much more clear cut. Serum viscosity was minimal at a preheat temperature of 60-66°C. At higher temperatures the pectic enzymes were inactivated, at lower temperatures the enzymes were inhibited. In both cases this resulted in higher serum viscosity. Hand et al. (1955) concluded that the effect of preheating on gross viscosity was due to a softening of the crushed tomato tissues prior to finishing in addition to the preservation of the pectin in the serum. Whittenberger & Nutting (1957) presented results which support the theory that, besides enzyme inactivation, softening of the tissue is important in determining the effect of preheating. Unheated green tomatoes gave a juice low in gross viscosity and cell wall content. Whole green tomatoes

preheated to 88°C yielded a juice high in gross viscosity, cell wall and pectin content. When ripe fruits were used, differences in preheat treatment caused smaller differences in juice structure (cells were easily separable) and pectin content; however, differences in gross viscosity were significant. Twigg (1959) concluded that preheat temperatures above 93°C appear to allow for the extraction of more structural solids, probably due to a softening effect on the tomato tissue, but may also cause a decrease in average molecular weight of the pectic constituents and in this way negatively affect gross viscosity. Luh & Daoud (1971) and Luh et al. (1984 c) suggest that the increase in serum viscosity at higher preheat temperatures may be explained by the solubilization of insoluble pectin to the soluble form.

In addition to its effects on viscosity, preheating may also affect the retention of ascorbic acid (Luh & Daoud 1971, Sherkat & Luh 1976, 1977, Bel-Haj 1981), colour (Cooler 1962, Sherkat & Luh 1976, 1977, Luh & Daoud 1971) and flavour (Sherkat & Luh 1976, 1977).

### *Effect of holding before finishing on gross viscosity*

In industrial practice there is often some delay between the crushing or breaking of the tomatoes and heating the crushed tomatoes to the desired preheat temperature. As early as 1944, Kertesz & Loconti stated that a juice of the highest possible viscosity cannot be obtained when the inactivation of the enzymes is performed after breaking the tomatoes. They showed that the holding of finished juice at room temperature for only 30 sec. caused a drop in both gross and serum viscosity. Even in samples heated immediately after making the juice (within 2-5 sec.) there was some viscosity loss when compared to the viscosity of juice made from canned tomatoes. McColloch et al. (1952) therefore concluded that the action of enzymes is so rapid that a 100% retention of pectic substances is not obtained even under the best practical industrial conditions of rapidly preheating freshly crushed tomatoes. Results presented by several other authors seem to confirm this statement. Luh et al. (1954) prepared juices according to an activated cold break process. Tomatoes were dipped in boiling water for 2 min. and finished through a 0.8 mm screen. The temperature of the juice was approximately 45°C. After 2 hours the enzymes

were heat-inactivated at 100°C. These juices were much lower in gross viscosity, total pectin and AIS when compared to normal hot break juices. Cooler (1962) reported that crushing tomatoes at room temperature (± 30°C) followed by holding at this temperature and finishing at 90°C yielded juices much lower in gross viscosity than juices obtained after crushing, quick heating to 90°C and finishing at 90°C. Miers et al. (1967) studied the possibility of inactivating pectolytic enzymes by the addition of acid. They found that a delay of 5 seconds in the addition of acid to macerated tomatoes already resulted in a sharp gross viscosity drop.

In contrast to these authors, Hand et al. (1955) showed that holding chopped tomatoes for several hours at temperatures of 21°C and 54°C before heating caused no significant reduction in either serum or gross viscosity. However, holding finished, unheated pulp for one hour at 38°C resulted in gross viscosity and serum viscosity losses. They conclude that instead of using the terms "hot and cold break" it is sufficient to state whether the chopped tomatoes were preheated prior to finishing and to report the preheating temperature. According to Hand et al., the term "hot-break" has caused a great deal of confusion because of the implication that the necessary heating must be accomplished during or immediately after chopping in order to preserve the pectin in the juice.

Moreover, Smit & Nortje (1958) found that a delay of three hours at 21-27°C between breaking (screen 6.4 mm) and preheating (82°C) resulted in higher gross viscosity at the same soluble solids content when compared to the gross viscosity of samples which were immediately preheated after breaking, even though pectin, methoxyl content and comparative serum viscosities were lower. As the WIS content was very much the same they suggested that the insoluble particles were structurally different. Crandall & Nelson (1975) studied the effects of holding on the viscosity of juice and puree prepared from two tomato varieties. Crushed tomatoes (screen 9.5 mm) were held at 21°C for 1 hour before heating to 93°C. For one variety, holding had no effect on the serum and gross viscosity of the juice, but the gross viscosity of the puree increased; the other variety showed lower serum viscosity of juice and puree after holding, but the gross viscosity of juice and puree increased. Leoni et al. (1979, 1980, 1981) presented results which indicate that holding cold break crushed

tomatoes at room temperature for up to 3 hours before finishing at 60-62°C may increase the gross viscosity of tomato concentrate. It was found that as a result of this holding, the hexametaphosphate soluble pectin increased while the sodium hydroxide soluble pectin remained fairly unchanged and the free galacturonic acid decreased when compared to a cold break without holding. The phenomenon was explained by assuming that without holding, PE and PG exhibited optimal activity during break (60-62°C) and evaporation (48-50°C). With holding, the breakdown of pectin is slower and obviously the saponified pectin precipitates as calcium-pektinates rather than being broken down further by PG. Holding was found to have a favourable effect on the gross viscosity of concentrates obtained from varieties with a high initial content of pectic substances (varieties for mechanical harvesting) whereas it scarcely affected gross viscosity in the case of conventional varieties. It has to be mentioned, however, that hot break concentrates were always higher in gross viscosity than either the cold break, or the cold break with holding, concentrate. The hot break concentrate contained much more water and sodium hydroxide soluble pectin, but less free galacturonic acid.

All the literature cited above deals with the effects of holding crushed tomatoes at relatively low temperatures. It can be concluded from this literature that the effects of holding are influenced by coarseness of the particles in the crushed tomatoes (small particles favour enzyme action), temperature of holding, duration of holding and probably by variety (amount of pectolytic enzymes). When, for example, the crushing of tomatoes results in coarse pieces (larger than 1 cm) and the temperature during crushing is kept at a low level (20°C), the moment of heating is probably less important. However, the heating rate should be as high as possible because, as Kertesz & Loconti stated in 1944, in the heat inactivation of any active system of enzyme and substrate the temperature passes through a range where the enzyme activity is the very highest.

The literature also offers some information on the effects of holding crushed tomatoes at higher temperatures, on juice gross viscosity. Luh & Daoud (1971) and Garces & Luh (1972) indicated that holding crushed tomatoes (3 mm screen) at temperatures insufficient for complete enzyme inactivation may result in decreased viscosities; moreover, they showed that holding crushed tomatoes at temperatures of 93°C, 104°C and 115°C

before finishing increased gross viscosity. However, the holding effect was smaller than the effect of an increased preheat temperature. Better pectin retention as a result of quicker enzyme inactivation served as an explanation for the increased gross viscosity. Miers et al. (1970) reported that crushed tomatoes yielding juices with high gross viscosity showed gross viscosity gains of up to 20% after 10 min. at 100°C but could show losses of up to 25% after 30 min. at 100°C. When initial gross viscosity was at a very low level, the juices from the heated crushed tomatoes showed increased gross viscosity even after 30 min. at 100°C. Increases and decreases in gross viscosity were smaller at 71°C holding. The viscosity of serums usually declined progressively through the 30 minutes heating period. The gross viscosity increase was explained by the softening of tomato tissue in the crushed tomatoes as a result of cooking; more solid tissue went through the pulper screen into the juice upon pulping. This increase in total solids was accompanied by a decrease in the % of insoluble waste from the pulper.

### *Effect of the finisher operation on gross viscosity*

Most of the information on the effects of the finisher operation on the gross viscosity of tomato juice has been obtained from studies of Hand et al. (1955) and Moyer et al. (1959).

Moyer et al. (1959), studying the factors affecting the yield of tomato juice, showed that, excluding the losses suffered during washing and trimming, being a reflection of raw product quality, the greatest variation in yield occurred in the finisher operation where the skins and seeds are separated from the juice. Of three methods of finishing (revolving paddle, tapered screw and eccentric drum), the paddle finisher provided the widest range in yields, but also, under optimal conditions for high gross viscosity, the highest yields. Hand et al. (1955) found that the use of a paddle type finisher run at intermediate or high speed, resulted in higher juice gross viscosities than the use of a screw extractor. This result was confirmed by Bel-Haj (1981). He also showed that double extraction with a screw type extractor always yielded higher gross viscosities than single extraction, although the differences were not significant.

With a paddle type finisher the effects of finishing were closely related to paddle speed and preheating temperature (Hand et al. 1955, Moyer et. al 1959). An increase in paddle speed always resulted in higher gross viscosity regardless of the preheating temperature; the effects on serum viscosity were smaller. Not only the gross viscosity but also the yield were shown to be proportional to paddle speed. At a low paddle speed few suspended solids, most of which were spherical, were incorporated in the juice and the juice was of low gross viscosity. At a high paddle speed more suspended solids were incorporated in the juice. These solids were more elongated and the juice had a high gross viscosity. In contrast, Bel-Haj (1981) stated that paddle speed had no significant effect on gross and serum viscosity and on pectin content. In addition to its effect on serum pectin, preheating exerts a softening effect on the tomato tissue, which in turn has an important practical influence on the action of the finisher. Therefore, lowest yields and low gross viscosities were obtained when samples were prepared without preheat treatment, especially when they were finished at low paddle speeds. As early as 1944, Kertesz & Loconti noted that manufacturers finish cold break juices at temperatures of 60 - 70°C in order to increase the yield. Temperatures in the range of 60-72°C gave the highest yields but low viscosities when extraction was at low paddle speeds. Serum viscosities at these preheat temperatures appeared to be at a minimum and therefore the serum was easily separated from the insoluble material, resulting in high yields; however, gross viscosity remained low as a result of the lack of suspended solids in the juice. In fact, the gross viscosity of these samples was lower than that of samples which were not preheated. When tomatoes were preheated to 93°C or higher, serum viscosity increased which made finishing at low paddle speeds more difficult. The yield of tomato juice dropped and gross viscosity was only slightly higher than gross viscosity obtained at intermediate preheating temperatures. At the high preheating temperatures, paddle speed had to be increased to maintain satisfactory yields; as the yield increased, so did the gross viscosity (Hand et al. 1955, Moyer et al. 1959). These results are in agreement with those of Cooler (1962) who found that steam blanching tomatoes reduced the yield. He suggested that serum viscosity is increased by the "in situ" enzyme inactivation and that this high viscosity serum

probably sticks to the particles. Whittenberger & Nutting (1957) also showed some results which stress the importance of preheating in determining the finishing effect. Unheated green tomatoes yielded a juice low in gross viscosity and cell wall content while unheated ripe tomatoes (cells more easily separable) yielded higher gross viscosity. Preheating at 88°C changed the picture completely; juices of green tomatoes were much thicker than those of red ripe tomatoes.

In general, preheating and finishing temperatures are approximately the same; however, it was found that the gross viscosity of juice decreased as a result of a decrease in finishing temperature after a preheat treatment at 93°C. Gross viscosity increased when juice was finished at 93°C after preheating at 77°C. However, the effect of an increase in preheat temperature was much larger than the effect of a finisher temperature increase (Hand et al. 1955). Cooler (1962) showed that finisher temperature (30°C or 90°C) had no influence on the gross viscosity of juices when tomatoes were crushed at room temperature (30°C).

Both Hand et al. (1955) and Moyer et al. (1959) observed increased gross viscosity and yield as a result of enlargement of the finisher screen openings (0.6, 0.8, 1.1, 1.5 mm). However, they stated that these increases were of no practical significance because tomato juice prepared using screen openings larger than 0.6 mm showed defects due to the appearence of specks consisting chiefly of seed fragments. Smit & Nortje (1958) found that the use of larger screen openings in a screw extractor (0.5-1.0 mm) increased paste gross viscosity, reduced serum separation but had little effect on serum viscosity. Twigg (1959) observed that the use of a 0.6 mm finisher screen resulted in ketchups lower in gross, serum viscosity and solids content but higher in serum separation, than ketchups prepared with a 0.7 or 0.8 mm finisher screen size. Bel-Haj (1981) found significant differences in juice gross viscosity between the use of a 0.7 mm and 0.8 mm screen opening. However, this difference was not accompanied by significant differences in soluble solids, total solids, total pectin or serum viscosity.

Moyer et al. (1959) showed that an inverse relationship exists between the % of finisher waste and the gross viscosity of tomato juice at a given preheating temperature. High yields are obtained when the finisher is adjusted to give a relatively dry waste material. Low yields result from

loss of the aqueous phase, or serum, which is obvious when one considers the fact that 99% of the juice by weight is serum. The conditions of finishing which conserve the greatest amount of serum also result in the highest % of suspended solids.

From the literature it seems that preheat treatment, type of finisher as well as finishing adjustments have an influence on the finishing effect. Finisher adjustments studied in literature were paddle speed, screen size and temperature. It seems reasonable, however, to assume that other factors, such as twist or pitch of the paddles, clearence between paddles and screen (Moyer et al. 1951) and juice input will have their influence on the finishing effect.

### *Effect of concentration on gross viscosity*

Industrially, tomato juice is often concentrated either for direct use in products as ketchup and other sauces (low solids concentrates) or for storage purposes (high solids concentrates). Theoretically many techniques are available for the concentration of tomato juices, for example: evaporation either at atmospheric pressure or under vacuum (Feinberg 1967), reversed osmosis (Merson et al. 1980, Ishii et al. 1981), the centrifuge separation, serum evaporation method as described by Sulc & Ciric (1968) or even freeze concentration (Deshpande et al. 1984). To date, however, commercial tomato concentrates are mainly prepared by evaporation; many evaporators and paste producing processes have been described in the literature (Randall et al. 1966, Carlson et al. 1967, Anon. 1975, Chen et al. 1979, Anon. 1981, Anon. 1981 b, Le Maire 1981, Ladwig 1982, Skierkowski 1982, Dale et al. 1982, Moresi & Liverotti 1982, Rumsey et al. 1984).

The method and degree of evaporation, as well as storage conditions, are important factors influencing the quality of tomato paste and concentrates. Quality can be evaluated on the basis of vitamin C content, degree of browning, colour, flavour, taste and gross viscosity. Lower evaporation temperature and shorter processing time are thought to minimize the heat damage to tomato concentrates and to improve quality (Mannheim & Kopelmann 1964). Within the scope of this thesis only the effects of

evaporation and some evaporation techniques on the gross viscosity of the concentrates will be discussed.

Kopelmann & Mannheim (1964) and Mannheim & Kopelmann (1964) studied the heat transfer coefficients of two evaporators (Luwa swept-surface and a atmospheric open-pan evaporator) used in tomato juice concentrate manufacture for two methods of juice evaporation (whole juice and serum evaporation); they also studied the effects of evaporation on juice quality. Juice was prepared by breaking at 60°C, holding for 5 min. and finishing through a 0.8 mm screen. It was reported that the serum evaporation method gave considerable savings in evaporation time. The juice was separated into fibres and serum and the serum was concentrated before resuspending with the fibres. This finding was expected to favour improved product quality. However, it was found that Bostwick values of 22°Bx paste samples were 5-6 cm for the juice evaporation method and 12-13 cm for the serum evaporation method, this irrespective of the evaporator type. The Blotter values (for description of this method see section 2.4.) were also much lower for the juice evaporation method. Kopelmann & Mannheim (1964) and Mannheim & Kopelmann (1964) showed that, after dilution of the pastes to 5°Bx, gross viscosities (Brookfield) for the juice evaporation method evaporated with the Luwa were twice as high as those for the serum evaporation method or the atmospheric evaporator. In general, it was found that the diluted paste samples had low gross viscosities when compared to commercial juice samples. The low gross viscosity for the serum evaporation concentrates was explained by referring to the results of Ephraim et al. (1962). They reported that centrifuging a cold break juice resulted in the crushing of cells and in disruption of the solid suspension structure of the juice, causing a gross viscosity loss of 50%. Heat damage as a result of prolonged heating at a high temperature may serve as an explanation for the lower viscosities of the juice reconstituted from the "atmospheric evaporation" paste in comparison to those of the juice from Luwa paste (Harper & El Sahrigi 1965).

Harper & El Sahrigi (1965) studying the viscometric behaviour of hot break tomato concentrates prepared in a conventional vacuum pan reported that dilution of a 30% TS paste to 25% TS or 16% TS resulted in a 20% gross viscosity loss when compared to the gross viscosity of concentrates at a corresponding solids level. They explained this finding by assuming some

experimental error. Dilution of a 30% TS reconstituted paste obtained by the serum evaporation method resulted, on the other hand, in gross viscosity losses of approximately 70% when compared to the original vacuum pan concentrates of the same solids content.

Marsh (1977, 1982) reported that the more water was required to dilute a sample (Pfaudler swept-film evaporator) to 12°Bx the higher was the Bostwick value reported for the product and the further it deviated from the value before concentration. A pasteurizing heat treatment followed by storage caused an increase in this effect. The extent of desiccation of the water insoluble fraction of the solids and their inability to resorb maximally is proposed as the cause of the phenomenon. The mechanism of resorption was partially restored following a heat treatment of the diluted paste (30 min. 100°C). A compilation of the results of Marsh et al. 1982, 1983 and 1984 showed a good correlation (r=0.92) between the true Bostwick value at 12°Bx and the Bostwick of a 12°Bx, 80°C heated dilution of a high solids paste. Tomato puree at 12°Bx with a Bostwick of 2-6 cm (30 sec, 20°C) will lose 1-2 cm Bostwick reading as a result of concentration to a high solids paste (30%TS).

Luh et al. (1984 b) found that total pectin (dry basis) and serum viscosity decreased during concentration and subsequent pasteurization. They state that in the industrial remanufacture of tomato products from pastes, such as ketchup and sauces, there is always some loss of gross viscosity in the reconstituted products. The phenomenon may be partially explained, according to Luh et al. (1984 b), by the change in size and solubility of the pectin and cellulose polymers due to the concentration and heating processes.

### *Effect of homogenization on gross viscosity*

It is clear from the previously discussed literature that a tomato juice containing large quantities of suspended matter will have a high gross viscosity. However, it is likely that gross viscosity is not governed by the quantity of cellular material alone, but also by the configuration or shape of the particles. It is well known that elongated particles have a greater effect on gross viscosity than more spherical particles. (Hand et

al. 1956, Moyer et al. 1957, Whittenberger & Nutting 1957). Several authors have shown that mechanical treatment may increase gross viscosity. In general, these increases in gross viscosity are attributed to a decrease in cell size, a change in cell shape (more elongated or linear) and an increase in total surface area. However, the effect of homogenization seems to depend largely on the method of juice preparation, the type of homogenizer and the method of gross viscosity measurement, causing the data from the literature to be somewhat confusing. Therefore, literature on the effects of homogenization is presented here in chronological order.

Luh et al. (1954) found that homogenization with a laboratory homogenizer increased the gross viscosity of hot break (disintegration of tomatoes in boiling water for 15 min.) tomato paste and puree.

Hand et al. (1955) presented photographs illustrating the positive effects of partial and complete homogenization with a piston type homogenizer on gross viscosity. Partial homogenization resulted in fragmentation of the predominantly spherical cellular particles; photographs of a completely homogenized juice show a brush-heap type of structure contributing to the increased gross viscosity.

Robinson et al. (1956) studied the factors influencing the degree of settling or serum separation in tomato juice. Homogenization reduced the average particle size but increased the volume of particles after centrifuging. The increase in volume was associated with the rupture of the cellular envelope. The fragmented cells did not settle as compactly as the intact cells.

Whittenberger & Nutting (1957) dissected tomatoes into four separate tissue fractions which were converted to juices. The juices containing cell walls (juices from shell tissue, center tissue and seed envelopes) were thickened by increasing the linearity and surface area of the walls through homogenization in a Waring blender. Although enzymatic digestion of pectin in a juice lowered gross viscosity, gross viscosity was restored by mechanically changing the structure of pectin free cell walls. Partial enzymatic digestion of cellulose in the cell walls irreversibely lowered gross viscosity. However, maximum gross viscosity was obtained by treating the original pectin containing juice in the blender. Evidently the combined effects of dissolved pectin in raising the serum viscosity and of insoluble

pectin in raising the water holding capacity of the fragmented cell walls, more than offset the greater wall fragmentation in the pectin free juice. The viscosity of the serum remained almost constant during the blender treatment. Whittenberger & Nutting (1958) showed that both hot and cold break juices thickened upon homogenization (2 min. Waring blender). Washed juices and cellulosic cell walls were also susceptible to mechanical break-up; in most cases the removal of electrolytes accentuated the thickening effect of homogenization. However, a hot break juice sample that thickened most upon washing did not increase further in thickness upon homogenization. Microscopic examination showed that most of the cell walls remained whole and unbroken. Apparently in this case the relatively large quantities of pectic substances in the walls acted as plasticizers and rendered the cell walls less brittle and more resistent to mechanical stress.

Ephraim et al. (1962) showed that mild or drastic homogenization of unheated hand strained juice resulted in great gross viscosity increases of 60-70% as result of the production of more particles of smaller size. Becker et al. (1972) stated that in cold break juices (delay of 1 hour between maceration in a Waring blender at low speed and heating to 95°C) the plant enzymes are able to attack pectin and other cell wall binding polymers. Degradation of these cementing polymers frees the cell wall fibers, which on blending (Waring blender, high speed) become entangled and raise the gross viscosity. Since the cold break juice lacks the dispersing effects of these polymers there is only a moderate gross viscosity increase. In hot break juices (macerate brought directly in water of 93°C, enzyme inactivation within 9 sec.) the enzymatic digestion of the binding polymers is prevented and the cell wall cement preserved. Therefore the cell wall fibres remain entwined and the cell is generally impervious to blending. Treating hot break juices with acid solubilizes protopectin from the cell walls, increases serum viscosity and decreases the % of intact cells. The cell wall, rid of its intercellular cement, disintegrates on blending into a "brush heap" of fibrils. These strands become entangled with strands from neighbouring brush heaps and juice gross viscosity increases several times. The viscous serum helps keep the brush heaps distributed throughout the solution. The serum soluble viscosity component liberated by the acid treatment must tend to remain entrapped in the brush

heaps until blending occurs. It then escapes to increase the serum viscosity. Individually neither the serum soluble polymers nor the cell wall fibers can cause gross viscosity changes. When a juice contains both large amounts of dispersed cell wall fibers and a high viscosity serum, the gross viscosity is greatly elevated. "Slow hot break" juices (heating macerate to 93°C within 45 sec.) gave slight increases in gross viscosity upon homogenization.

Crandall & Nelson (1975) showed that mechanical treatment (commercial high speed rotary mill) decreased gross viscosity and serum viscosity of the juice prepared by the boiling break method (tomatoes 45 min. in boiling water before crushing). The gross viscosity of "modified hot break" juice (macerate heated to 93°C within 2 min.), measured by efflux, is increased by mechanical treatment. The efflux measurements of the modified cold break juice (delay of 1 hour between maceration and heating to 93°C) showed that mechanical treatment had only a slight positive effect on gross viscosity. Serum viscosity of the modified hot- and cold break juices are not significantly changed when mechanically treated. Bostwick measurements of puree samples (15°Bx) show significant increases in gross viscosity after homogenization for the boiling break and the modified hot break method; modified cold break puree did not react upon homogenization. When puree samples were diluted and measured with the efflux pipette it was found that all samples, except the boiling break puree of one variety, decreased or remained unchanged in gross viscosity after homogenization.

In general literature shows that the gross viscosity of all types of tomato juice increased upon homogenization when the right type of homogenizer was chosen. There are indications that cell walls containing large amounts of pectin are less brittle and need more power or pressure to be homogenized than cells of which the pectin has been removed by acid or pectolytic enzymes. However, once homogenized, the pectin containing walls raise gross viscosity more than walls devoid of pectin. Concentrates, probably due to the higher amount of cells present, seem to respond more quickly to homogenization than do juices.

*Effect of additives on gross viscosity*

The addition of compounds such as acid, salts and sugars to tomato juices and products has sometimes been reported as influencing viscosity.

Acid has been added to tomato products at three stages of processing: 1) before heat treatment, for the purpose of enzyme inactivation (Wagner & Miers 1967), 2) to juice, for the purpose of acidified bulk storage (Dougherty & Nelson 1974, Sidhu et al. 1984) and 3) in the formulation of ketchups and other sauces.

Wagner et al. (1969) observed increases in gross viscosity when acid was added prior to the heat treatment. They attributed the increases to quicker enzyme inactivation, increased extraction of pectin and improved dispersion of cell wall components. Crean (1969) found that the extractibility of pectin from cell wall materials was not significantly affected by pH, and hence concluded that changes in gross viscosity were caused by physical changes in cell wall materials and soluble pectins. The effect was reversible; the original gross viscosity could be completely restored by readjusting the pH to its original value. Takada & Nelson (1983) reported that viscosities of a model system of tomato product (citrus pectin + bovine serum albumin), of diluted tomato puree and of 10°Bx tomato puree were affected by pH changes. Maximum viscosity was obtained at pH 4.2-4.4, the original viscosity was restored after readjusting the pH. They explained these findings by assuming a reversible electrostatic complex formation between pectin and protein.

Dougherty & Nelson (1974) and Sidhu et al. 1984) stated that, in bulk storage, the acidification of tomato juice would reduce the required heat treatment and aid in potential spoilage control during storage. Dougherty & Nelson (1974), however, found decreases in juice gross viscosity when tomato juices were stored at pH levels from 2 to 4 for a period of up to 3 months. These gross viscosity losses were irreversible and were not caused by the net addition of NaCl (0.7% when acidified to pH 2) after neutralization of the juice to pH 4.3. Hydrogen bonding and other associations among pectins, celluloses and/or other constituents could have been permanently disrupted as a result of acidification. (Dougherty & Nelson 1974). Gross viscosity of cold break juices was not affected by storage for 3 months under acid conditions (pH 1.3-1.4). Acidified juices

showed a slight off-flavour after bringing pH to 4.45; however, this was not detected in ketchups prepared from acidified cold break juices (Sidhu et al. 1984).
Twigg (1959) detected increases in ketchup viscosity with a decrease in pH, and contributed this to increases in the viscosity of low ester pectins.

Results presented by Whittenberger and Nutting (1957, 1958) indicate that the cell wall surfaces are electrically charged, helping to maintain walls in suspension. The addition of 0.2% sodium chloride and calcium chloride to washed cell wall material dampened the charges considerably, causing decreased viscosity. Twigg (1959) found that the addition of approximately 8.5 g/l calcium chloride to tomato juice increased ketchup gross viscosity and decreased serum separation. The values for water soluble pectin were greatly reduced, while the amounts of oxalate soluble pectin greatly increased. Cooler (1962) showed that calcium chloride increased the gross viscosity of steam blanched tomato juice but had no effect on cold break juices. Magnesium chloride decreased the gross viscosity of the steam blanched juice. He concluded that electrolytes are important secondary agents imparting electroviscous properties to macromolecules promoting thickening by extension and thinning by contraction. Dougherty & Nelson (1974) did not detect any decrease in juice gross viscosity as a result of the addition of 1% sodium chloride. Sherkat & Luh (1977), however, presented results which clearly show that the addition of 0.8% sodium chloride to reconstituted 5°Bx juice, or 1.6% sodium chloride to reconstituted sauce of 12°Bx, resulted in lower gross viscosities due to the decrease in the hydration of cellulose, pectin, proteins and other polymers.

Bel-Haj (1981) reported in his literature review that the apparent viscosity of pectin solutions is increased by the addition of sugars such as glucose, maltose and sucrose. The increase in viscosity is attributed to the formation of H-bonds and aggregates of sugar-pectin molecules, as well as to the dehydrating effect of the sugar.

### *Effect of additional heat on gross viscosity*

Usually tomato products receive a heat treatment, before or after

packing, in order to prevent spoilage by microorganisms. This additional heat treatment, however, may affect the viscosity of the tomato product.

Miers et al. (1970) stressed that processors should keep heating to a minimum once the crushed tomatoes are finished, especially when they adapt techniques by which products having a high viscosity are produced. Juices having high gross and serum viscosities lost 35-65% of the serum viscosity and 10-18% of the gross viscosity after 10 min. heating at 100°C. Viscosity losses were lower at lower temperatures and with juices having lower initial viscosity. Stephens et al. (1970) detected gross viscosity losses of 10-20% after 10 min. heating at 88°C; especially high viscosity juices were heat-sensitive. Hand et al. (1955) found no differences in juice gross viscosity after pasteurization at 93, 121 or 129°C. However, they do not mention the gross viscosity before pasteurization.

Miers et al. (1970) explained the loss in viscosity by hydrolysis or other types of degradation of the polymeric components present in the tomato juice both in the serum and the insoluble fraction. Miers et al. (1970) present extensive literature data indicating that the at random cleavage of a limited number of bonds in a high molecular weight pectin may result in a viscosity loss of over 50%.

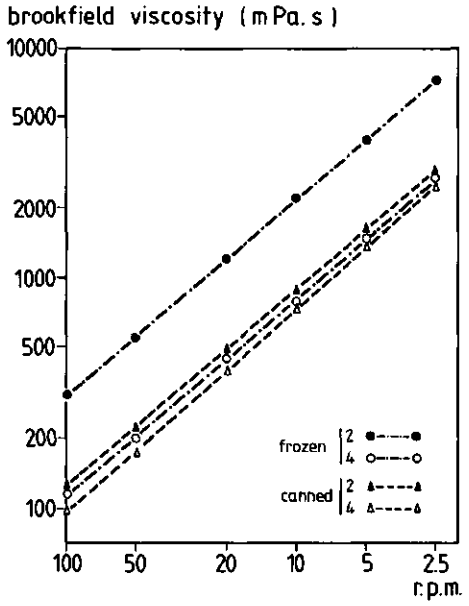



Fig. 9: Effect of pasteurization on gross viscosity of tomato juices reconstituted from paste.
The hot break temperature was 100°C for sample 2 and 64.4°C for sample 4 (from Sherkat & Luh 1977).

They conclude that tomato products having high viscosity and high molecular weight pectins could lose gross viscosity very quickly as a result of heat by the cleavage of only a few bonds. Indeed, Luh et al. (1984a) found a definite decrease in weight average molecular weight of pectinic acids and often of pectic acids after hot break (40 sec. 107°C), concentration (30 min. 45°C) and pasteurization (30 min. 100°C).

Sherkat & Luh (1976, 1977) showed that heat processing of 26°Bx paste for 30 min. in boiling water resulted in significantly lower gross viscosity after dilution to 5°Bx when compared to samples frozen at -23°C. The negative effect of pasteurization was much higher for samples having a preheat treatment at 100 °C than for samples preheated at 64°C. (Fig. 9). Heat processed pastes contained lower total pectin and protopectin contents than frozen pastes.

*Effect of storage on gross viscosity*

The data on the influence of storage time and storage temperature on the gross viscosity of tomato products are rather limited. Cooler (1962) found that hot break juices thinned during storage while cold break juices were not affected. He assumed that hot break juices existed as weak gels, which seldom attain an equilibrium state. Mannheim & Kopelmann (1964) reported that the gross viscosity of cold break paste did not change during a storage period of 14 weeks; there was no difference between storage at room temperature or storage at 37°C. Marsh et al. (1984) studied the effects of storage on the gross viscosity and solids content of hot break tomato juices and intermediate level concentrates prepared from 12 varieties. Storage of up to 18 months, at ambient temperature, had no distinct effect on total solids, soluble solids and WIS/TS ratio of the juice. Serum viscosity was found to decrease while gross viscosity (Bostwick) tended to increase slightly. Storage had almost no influence on the Bostwick viscosities of intermediate level concentrates (10-15°Bx). Marsh et al. (1982) presented results which show that the gross viscosity of 12°Bx dilutions of high solids pastes decreased as a result of storage of the paste for 9 months at ambient temperature; gross viscosity of the corresponding juice samples on the other hand increased slightly as result of storage.

## 2.4. *Factors affecting serum separation in tomato products*

The degree of serum separation, which is the tendency of the insoluble solids to settle out, leaving a practically clear liquid at the top of the tomato product, is considered to contribute to the overall term consistency. For tomato juice and tomato ketchup, consistency is an important parameter in determining the quality grade. Serum separation is often measured by determining the Blotter value (distance of travel of serum in filter paper measured from the edge of a mound of tomato product), the degree of settling (volume of serum at the top in a graduated cylinder as % of the total volume) or the rate of filtration (volume of filtrate obtained after filtering a certain amount of tomato product).

Robinson et al. (1956) reported that, as a general rule, there is an inverse relationship between the degree of settling and the gross viscosity of tomato juice. They state that the phenomenon of settling in ordinary tomato juice is not a simple process of sedimentation but involves a closer packing together of particles in contact with each other. In this context the shape of the particles seems to be more important than average particle size. It was found that the degree of settling is determined by the amount of insoluble solids in suspension and by the extent to which the intact cells are disrupted. Rupture by homogenization reduced the degree of settling. The amount of total pectin in tomato juice did not have a major effect on the degree of settling.

The formation of a clear serum in the uppermost region of the juice column can be explained by two distinct processes (Shomer et al. 1984): 1) too much liquid, for which a given concentration of insoluble particles cannot fill the volume of the liquid column; this may occur within several hours of shelflife; and 2) a slowly diminishing volume of the precipitate during storage as a result of gradual collapse under gravity stress. Shomer et al. (1984) confirmed the conclusion of Robinson et al. (1956) that serum has no effect on the settling of the particles. Intact or disrupted cell walls from tomato pericarp could not be suspended in the juice, even when the viscosity and/or density of the serum was increased by the addition of high esterified pectin and sucrose. Hence they considered tomato juice to be a swelled precipitate rather than a suspension. Pectinase addition

resulted in an increased precipitate volume due to swelling of the wall and partial dispersion of the microfibrilar system. Cellulase addition led to partial or complete degradation of the microfibrilar system with a resultant collapse of the precipitate. Homogenization increased the volume of the precipitate. The addition of pectinase after homogenization gave an additional increase, the addition of cellulase resulted in collapse to the level of intact cells treated with cellulase. Studies with light and electron microscopes led to the supposition that the cellulose microfibrilar system formed and supported the construction of the precipitate. Shomer et al. (1984) therefore concluded that the inactivation of pectinases is not the reason for an improved homogeneous appearence as a result of hot break. They suggested that if endogeneous enzymes affect the formation of a clear serum at the top of the juice column, these enzymes are apparently cellulases. The activity of cellulases during the ripening, postharvest and processing stages may decrease the ability of the microfibrilar system to withstand collapse under gravity stress. In contrast to the work of Shomer et al. (1984), Smit & Nortje (1958) found that the addition of Pectinol A to paste reduced gross viscosity and serum viscosity and increased the degree of settling. However, they did not take into consideration the fact that Pectinol A also contains cellulase activity.

Factors influencing the degree of serum separation in ketchup were studied by Twigg (1959). Neither gross viscosity nor serum viscosity correlated well with serum separation as measured with the Blotter-test. Multiple regression analyses with the four compositional factors: structural solids, non structural solids, total pectin and pH, yielded a correlation of 0.69 with serum separation. The relative influence of the four factors on explainable serum separation differences were: structural solids 57%; non structural solids 22%; pH 19% and total pectic substances 2%. All factors except pH had a negative relationship with serum separation. Cooler (1962) concluded that the Blotter-test measures the mobility of solution, which is related to serum viscosity, to the degree of entrapment within the suspended particles and also to the amount of serum relative to the suspended particles.

Marsh et al. (1979 b) showed that gross viscosity and serum separation were unrelated quality attributes in ketchup. When gross viscosity was

standardized to a given Bostwick level, serum separation varied from none to considerable. In contrast to all above cited authors they reported that serum separation depends on serum viscosity. Retention of at least 80% of the tomatoes' original serum viscosity is required for minimal serum flow. Components responsible for high serum viscosity apparently also control serum separation.

Increases in hot break temperature, finisher paddle speed and finisher screen size, as well as homogenization of the suspended particles, were found to decrease the level of serum separation in tomato juice and ketchup (Robinson et al. 1956, Smit & Nortje 1958, Twigg 1959, Cooler 1962). Storage of ripe machine harvested fruits, on the other hand, resulted in large serum separation increases. (Marsh et al. 1979 b).

## 2.5. *Composition of tomato polysaccharides*

In paragraph 2.3.4. of this literature review the importance of tomato polysaccharides as contributors to gross viscosity of tomato products was discussed.

Tomato polysaccharides are isolated by preparing alcohol insoluble solids (AIS) or water insoluble solids (WIS). The AIS cover more or less the complete polysaccharide content while WIS represent only the cell wall polysaccharides. Large differences are found in amounts of AIS and WIS in tomatoes and tomato products (Table 9); a result of differences in variety and degree of ripeness.

Complete chemical analyses of AIS or WIS from ripe tomatoes are rarely found in literature. Gross & Wallner (1979) and Gross (1984), analysing isolated cell walls of outer pericarp from ripe tomatoes, found high amounts of uronic acid, cellulose and galactose (Table 10). However, most authors use the complex of polysaccharides for fractionation and particularly for the isolation of pectin fractions. Gross (1984) for example fractionated cell walls in 22.7% ionically associated pectin (chelator soluble), 9.7% covalently bound pectin (alkali-soluble), 19.3% hemicellulose and 48.3% cellulose. Jona & Foa (1979) found that tomato cell wall pericarp was highest in pectic substances when compared to other fruits and vegetables as peach, pepper, strawberry, cherry, apple and grape. In general, the pectic substances were the main component of fruit

Table 9: % AIS and % WIS in tomato fruit and juice (fresh weight basis).

| % AIS | % WIS | Product | Reference |
|---|---|---|---|
| 1.3-1.9 % | | tomato | McColloch et al. 1950 |
| 1.96-2.04% | | tomato | Woodmansee et al. 1959 |
| | 0.66-0.90% | tomato | Stevens 1972 |
| 1.01-2.32% | | tomato | Janoria 1974, Janoria & Rhodes 1974 |
| 1.40-1.73% | | tomato | Stein & Brown 1975 |
| 1.20-2.71% | | tomato | Stevens & Paulson 1976 |
| 1.22-1.71% | | tomato | Brown & Stein 1977 |
| 1.2% | | tomato | Malis-Arad 1983 |
| 1.08-1.82% | | juice | Cooler 1962 |
| 0.80-0.85% | | juice | Wagner et al. 1969 |
| | 0.54-1.42% | juice | Marsh et al. 1979-1984 |
| | 0.79-1.07% | juice | Luh et al. 1982, 1984 |

Table 10: Composition of isolated cell walls of ripe tomatoes (% w/w).

| | Gross & Wallner 1979 | Gross 1984 |
|---|---|---|
| Rham. | 1.5 | 1.2 |
| Ara. | 2.7 | 3.0 |
| Xyl. | 3.4 | 4.3 |
| Man. | 2.3 | 2.4 |
| Gal. | 6.3 | 4.0 |
| Gluc. | 2.2 | 2.2 |
| AGA | 30 | 25.4 |
| Cellulose | 30.0 | 46.1 |
| Protein | 3.8 | -- |
| | 82.2% | 88.6% |

cell walls, followed by cellulose, while hemicellulose accounted for only a small fraction of the total polysaccharides. For tomato fruit cell walls Jona & Foa (1979) reported a composition of 51% pectin, 18% hemicellulose and 31% cellulose.

*Pectin*

Table 11 shows a compilation of literature data on the pectin content in ripe tomato fruit and tomato juice. Total pectin content ranges from 0.2-0.5% on a fresh weight basis, which will equal 3.0-7.5% on a dry basis. Luh et al. (1984 b, 1984 c) found that total pectin on dry basis decreased from 3.96-6.75% to 2.92-5.74% when juice was evaporated to paste and from 5.46-8.94% to 4.95-6.25% when tomatoes were processed to paste. Expressed as % of AIS, total pectin ranges from 14-26% in fresh tomatoes (Woodmansee 1959, Stephens et al. 1970, Stein & Brown 1975, Malis-Arad 1983). Stephens et al. 1970 showed that processing tomatoes into juice (hot break method) decreased this percentage from 18.1-19.2% for tomatoes to 10.8-13.3% for juice. Table 11 also shows a large diversity in the contribution of the different pectin fractions to the total amount of pectin. These differences are probably caused by dissimilarity in fractionation methods, although variety and ripeness level certainly play a role.

Little information is available on the chemical composition of the pectin or the pectin fractions. Luh et al. (1954 a) determined the anhydrogalacturonic acid (AGA) content and degree of esterification (DE) of the pectin fractions in tomatoes. They found AGA values of 68, 78, 47 and DE values of 75, 42, 56 for water, oxalate and HCl soluble pectin respectively. Other DE values found in literature are 55 for water, 40 for oxalate and 35 for HCl soluble pectin (Becker et al. 1968). The work of Brown & Stein (1977) indicates that galactose is the most important neutral sugar in the pectin fractions (approximately 50% of total neutral sugars). Other neutral sugars analysed were xylose, arabinose, ribose and rhamnose; no significant differences in the composition of the pectin fraction between cultivars were found. The composition of the ionically associated

Table 11: AGA content in fresh tomatoes and tomato juice (%w/w).

| reference | product | AGA total | AGA soluble in: | | | | | insoluble AGA |
|---|---|---|---|---|---|---|---|---|
| | | | $H_2O$ | oxalate | EDTA | HCl | NaOH | |
| Luh et al. 1954a | tomato | 0.13-0.24 | 0.01-0.04 | 0.07-0.15 | | 0.05 | | |
| Stier et al. 1956 | tomato | | 0.03 | 0.05 | | 0.11 | | |
| Foda 1957 | tomato | 0.33-0.43 | 0.10-0.15 | 0.09-0.13 | | 0.08 | | |
| Woodmansee 1959 | tomato | 0.26-0.34 | 0.17-0.26 | | | | | |
| El Sayed & Ericksen 1966 | tomato | 0.44-0.54 | 0.28-0.32 | | 0.16-0.20 | | | |
| Stein & Brown 1975, 1977 | tomato | 0.37-0.39 | 0.17-0.18 | 0.07-0.08 | | 0.04 | 0.07-0.09 | |
| Malis-Arad 1983 | tomato | 0.30 | 0.07 | | 0.20 | | | 0.03 |
| Luh et al. 1984 b,c | tomato | 0.36-0.46 | | | | | | |
| Luh et al. 1960 | juice | 0.24 | 0.18 | | | 0.06 | | |
| Becker et al. 1968 | juice | | 0.07 | 0.01 | | 0.02 | | |
| Luh & Daoud 1968 | juice | 0.36-0.47 | | | | | | |
| Luh et al. 1982 | juice | 0.22-0.35 | | | | | | |
| Luh et al. 1984 b | juice | 0.29-0.48 | | | | | | |

pectin (I.A.P.) and covalently bound pectin (C.B.P.) as determined by Gross (1984) is shown in Table 12. The most important neutral sugars in these pectin fractions are arabinose, galactose and rhamnose.

Table 12: composition of two cell wall pectin fractions (% w/w).

| | I.A.P. | C.B.P. |
|---|---|---|
| Rham. | 1.8 | 1.6 |
| Ara. | 3.8 | 3.3 |
| Xyl. | 0.5 | 0.8 |
| Man. | 0.2 | 0.2 |
| Gal. | 2.0 | 1.6 |
| Gluc. | 0.5 | 0.3 |
| AGA | 69.8 | 15.9 |
| total | 78.6% | 23.7% |

It is obvious that only a small part of the C.B.P. fraction is actually pectin.

Luh et al. (1984 a, 1984 c) established molecular weights of pectins by viscosity measurements. They report weight average molecular weights of 7,870-26,800 daltons for pectinic acids and 6,600-24,000 daltons for pectic acid, depending on variety. The processing of tomatoes to paste decreased the molecular weight of pectins. Stein & Brown (1975) report a molecular weight of $>2 \times 10^5$ for all the pectin fractions as determined with gel filtration. These values, however, are probably overestimated due to charge effects as a result of elution with water.

### *Hemicellulose*

Generally hemicellulose is solubilized from depectinized AIS, WIS or cell wall material by the addition of concentrated alkali (4 M). Herranz et al. (1981) report a hemicellulose content in the edible part of fresh ripe

tomatoes of 2.06% ± 1.06% on dry basis and 0.13% ± 0.07% on fresh weight basis. Gross (1984) found a hemicellulose content of 17.2% after fractionation of cell wall material. From findings of Williams & Bevenue (1954) it can be calculated that hemicellulose approximates 4.0% on dry basis and 21% on AIS basis. In their study Gross & Wallner (1979) conclude that hemicellulose is not degraded during tomato fruit ripening; however, Huber (1984) clearly shows that the hemicellulose of ripe fruits contains lower quantities of high MW polymers and higher quantities of low MW (<40,000) polymers when compared to the hemicellulose of unripe fruit.

Williams and Bevenue (1954) found the hemicellulose fraction to consist of 37.5% xylose and glucose containing polymers and 15% protein while 47.5% was not identified. The hemicellulose fraction isolated by Gross (1984) contained 21.9% glucose, 19.5% xylose, 8.4% mannose, 4.3% galactose, 4.0% arabinose and 4.3% anhydrogalacturonic acid, leaving 37.6% for unidentified components. Holloway & Greig (1984) present relative compositions of hemicellulose fractions of tomato and other fruits and vegetables (Table 13).

Table 13: Relative composition of hemicellulose of several fruits and vegetables.

| | Rham. | Ara. | Xyl. | Man. | Gal. | Gluc. | AGA | Methoxyl | Acetyl | Protein |
|---|---|---|---|---|---|---|---|---|---|---|
| tomato | 0 | 3.7 | 27.0 | 12.1 | 6.9 | 31.9 | 9.3 | 0.5 | 0.4 | 8.2 |
| pear | 1.6 | 17.2 | 19.8 | 3.8 | 13.4 | 27.7 | 8.3 | 0.7 | 1.7 | 5.8 |
| peach | 1.8 | 9.4 | 18.3 | 4.2 | 11.9 | 19.6 | 9.5 | 1.3 | 2.5 | 21.4 |
| cabbage | 1.8 | 14.3 | 18.0 | 7.5 | 31.4 | 22.9 | 16.9 | 1.3 | 3.9 | 11.6 |

Noteworthy are the low amounts of arabinose and galactose and the high amounts of mannose in the hemicellulose of tomatoes when compared to other fruits and vegetables.

*Cellulose*

The residue of AIS, WIS, or cell wall material left after solubilization of pectin and hemicellulose is considered to be the cellulose fraction. Values reported for the cellulose content in tomatoes and tomato juice are in the range 0.3-0.6% on fresh weight basis (Luh et al. 1960, 1982, 1984 b,c; Herranz et al. 1981; Southgate 1976). On the basis of cell wall weight, Gross & Wallner (1979) and Gross (1984) report values of 30.0% and 46.1% respectively. The cellulose fraction as isolated by Gross (1984) is composed of 68.5% cellulose, 11.9% non cellulosic neutral sugars and 0.4% anhydrouronic acid.

*Protein*

A fourth important component in AIS or WIS is protein, which is not isolated as a separate fraction but is probably included both in pectin and hemicellulose fractions. The amount of protein is generally calculated from the total nitrogen content as determined by the Kjehldahl method.
As has been shown before (Table 8), the protein content in AIS ranges between 20-30%. Gross & Wallner (1979), however, report that isolated cell wall material contained only 3.8% protein. This discrepancy is probably caused by a different method of sample preparation.

# 3. GENERAL ANALYTICAL METHODS

## 3.1. Viscosity measurements

### Gross viscosity

The gross viscosity of tomato products (juice and concentrates at several °Bx levels) was determined using the Haake rotovisco RV, a rotational viscometer, and the Bostwick consistometer, an instrument commonly used in the tomato industry. The Bostwick consistometer consists of a rectangular, graduated trough divided in two sections by a spring operated gate assembly. The smaller of the two sections serves as the reservoir for the material to be tested. Opening of the gate results in flow of the sample through the trough. Gross viscosity data measured with the Bostwick consistometer are reported as cm flow after 30 sec. at 20°C (Davis et al. 1954). When used for measuring gross viscosity the Haake rotovisco was equipped with the MV sensor system and the $MV_{III}$ rotary bob. Gross viscosity data were calculated from the torque signal S, given in scale graduations 0 to 100, using the formula: $\eta = \frac{G.S}{n}$ (mPa.s). In this formula G is a constant instrument factor and n is the rotor speed ($min^{-1}$) (Schramm 1981). Gross viscosity is reported as apparent viscosity ($\eta_{app}$) at 20°C using a rotor speed of 243 rpm.

Fig. 10 shows the relationship between the gross viscosity values as obtained with the Bostwick consistometer and the Haake rotovisco. This figure indicates that the Bostwick consistometer is not sensitive enough for measuring high viscosity tomato products. On the other hand, however, this instrument is very suitable for measuring the gross viscosity of tomato products with a Bostwick flow of 5 cm or more.

### Serum viscosity

Ca. 30 g. of tomato product was centrifuged for 20 min. at 48,000 x g. in a Sorvall RC-5B refrigerated superspeed centrifuge. The serum was filtered through Whatman GF/F (0.7 µm) and Schleicher & Schüll (0.45 µm) membrane filters. The viscosity of the serum was measured with the Haake rotovisco using sensor system NV, and expressed as mPa.s at a shear rate of 873.3 $sec^{-1}$ (20°C).

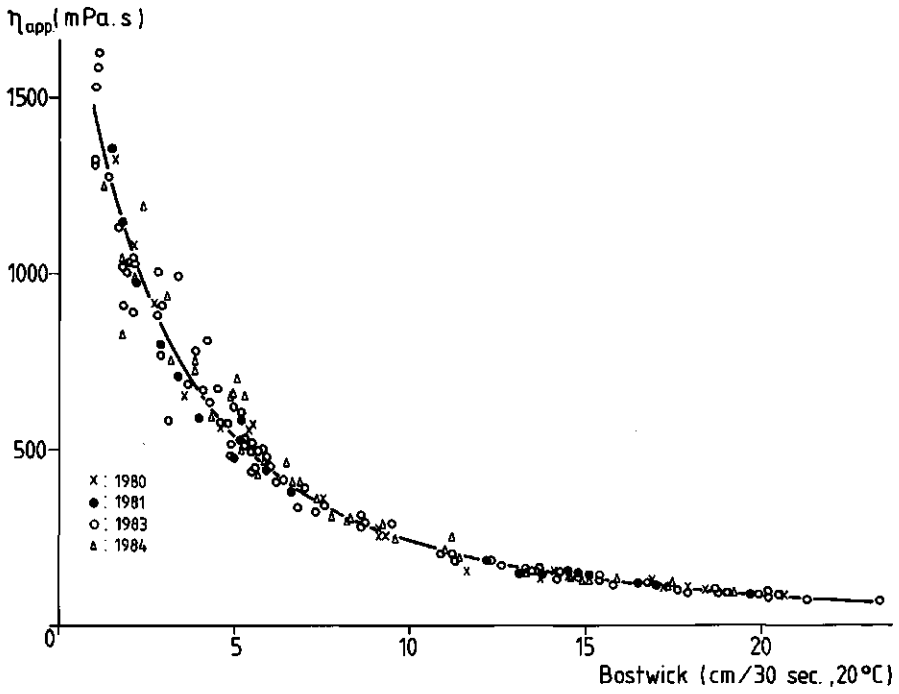



Fig. 10: Relationship between gross viscosity values measured with both the Bostwick consistometer and the Haake rotovisco.
Bostwick cm = 237.3 x $1/\sqrt{\eta_{app}}$ - 5.2 (n = 160, r = 0.995).

## 3.2. *Determination of solids content*

### *Soluble solids (°Bx)*

Ca. 30 g. of tomato product was centrifuged for 20 min. at 48,000 x g in a Sorvall RC-5B refrigerated superspeed centrifuge. After filtration of the serum through Whatman GF/F (0.7 µm) and Schleicher & Schüll 0.45 µm membrane filters, the amount of soluble solids in the filtered serum was determined by reading the sugar scale of an Abbé refractometer at 20°C.

### *Total solids (%TS).*

The total solids content of the tomato products was determined using the vacuum oven drying method described by Lamb (1977).

### *Preparation of water insoluble solids (WIS)*

Ca. 30 grams of tomato product was centrifuged for 20 min. at 48,000 g

in a Sorvall RC-5B refrigerated superspeed centrifuge. The residue obtained after decanting the supernatant was extracted for one hour with water of 50-55°C. Extraction and centrifugation were repeated until the °Bx in the supernatant was zero. After the last extraction, the residue was resuspended in a small volume of water, freeze-dried and weighed. After reducing the particle size of the WIS to ± 0.7 mm with a hammer mill (Culatti DFH 48), the WIS was stored at 3°C until further analysis. Fresh tomatoes were heated in a microwave oven to a heart temperature exceding 90°C. After cooling, the skins and seeds were removed by hand. The further procedure for the preparation of WIS from fresh tomatoes was identical to the procedure described above.

*Preparation of alcohol insoluble solids (AIS)*

Two volumes of 96% ethanol, containing 0.05 N HCl, were added to one volume of tomato product. After an extraction of one hour at room temperature (stirring) the mixture was filtered on a Büchner funnel under suction. The filtrate was discarded and the procedure was repeated once with 60-70% ethanol and three times with 96% ethanol. After the last extraction the residue was decoulorized with diethylether and air-dried. After reducing the particle size of the residue with a Culatti DFH 48 hammer mill to ± 0.7 mm., the AIS was stored at 3°C until further analysis.

*Preparation of serum-alcohol insoluble solids (s-AIS)*

Tomato juice was centrifuged for 45 min. at 25,400 g in a Sorvall RC-5B superspeed centrifuge. The serum was weighed and filtered through a D-1 glass sintered filter and then through Schleicher & Schüll glass microfibre paper no. 8. Two volumes of 96% ethanol were added and enough 37% HCl to make the solution 0.05 N. The precipitate was filtered through linen cloth, washed with 96% ethanol, dissolved in a small volume of distilled water and freeze-dried.

## *3.3. Chemical analysis of WIS*

*Neutral sugar analysis*

The neutral sugar composition was determined by glc after

hydrolysis of the polysaccharides to monomers and conversion of these monomers to alditol acetates, according to the method described by Jones & Albersheim (1972).

Several methods have been used for the hydrolysis of polysaccharides. 2 N TFA hydrolysis (Jones & Albersheim 1972) and 1 M $H_2SO_4$ hydrolysis (Englyst 1981) have been used for the determination of non-cellulosic polysaccharides and both are reported as giving comparable results (Englyst 1981). 0.8 N $H_2SO_4$ hydrolysis (Saeman 1963) and 2 N $H_2SO_4$ hydrolysis (Selvendran et al. 1979), both after prehydrolysis with 72% $H_2SO_4$, have been used for the determination of total polysaccharides.

Table 14 shows the effect of some methods of hydrolysis on the analysed sugar composition of tomato WIS.

Table 14: Sugar composition (% w/w) of tomato WIS in relation to method of hydrolysis.

| | | 1 hour prehydrolysis | | 3 hours prehydrolysis | |
|---|---|---|---|---|---|
| | 2 N TFA | 0.8 N $H_2SO_4$ | 2 N $H_2SO_4$ | 0.8 N $H_2SO_4$ | 2 N $H_2SO_4$ |
| Rhamnose | 1.0 | 0.2 | 0.4 | 0.2 | 0.5 |
| Arabinose | 1.3 | 1.2 | 1.1 | 1.2 | 1.1 |
| Xylose | 3.6 | 3.6 | 3.3 | 3.6 | 3.7 |
| Mannose | 1.5 | 2.7 | 2.8 | 2.7 | 2.9 |
| Galactose | 2.6 | 2.3 | 2.0 | 2.2 | 2.2 |
| Glucose | 2.2 | 20.9 | 26.6 | 27.1 | 28.7 |

The determination of the neutral sugar composition of tomato WIS after hydrolysis with 2 N TFA, results in underestimation of the mannose content. Therefore the method of determining the non-cellulosic polysaccharides in combination with determining cellulose cannot be used for tomato WIS.

In general, the 0.8 N $H_2SO_4$ and 2 N $H_2SO_4$ hydrolysis is preceded by a prehydrolysis with 72% $H_2SO_4$ for 1 hour at 30°C. Table 14 also presents results for the sugar composition of tomato WIS when the prehydrolysis is extended to 3 hours at 30°C. The main effect of an extension in

prehydrolysis time and an increase in $H_2SO_4$ normality is an increase in the determined glucose content. In comparison to the 2 N TFA hydrolysis, $H_2SO_4$ hydrolysis results in lower rhamnose, galactose and arabinose contents. The determination of the response factors of the sugars, relative to the internal standard, also shows that xylose is degraded when the prehydrolysis step is extended to 3 hours.

None of the methods of hydrolysis mentioned in Table 14 was able to hydrolyse the cellulose completely, as the determined cellulose content of this sample, according to the Updegraff method, was 32.1%. In order to increase the degree of hydrolysis of particularly the cellulose, the tomato WIS sample was prehydrolysed with an enzyme mixture prior to 2 N TFA hydrolysis. The enzyme mixture was prepared from desaccharified Ultrazyme 100 (Novo Ferment AG, Basel, Switzerland) and Maxayme CL 2000 (Gist Brocades, Delft, The Netherlands). Ultrazyme 100 was dissolved in 0.01 M sodium acetate buffer pH 5.0 and ultrafiltered in an ultrafiltration cell (Amicon Diaflo type 50) equipped with a YM 5 Diaflo filter. Maxazyme CL 2000 was dissolved in 0.01 M sodium acetate buffer pH 5.0 and desaccharified by gel filtration on a Bio-Gel P-10 column.(Bio-Rad Labs., Richmond, Cal., USA) Novo Ferment A.G. also provided us with the purified fermentation liquor from which Ultrazyme is prepared. This liquor lacks maltodextrins and therefore does not need desaccharification. Maxazyme CL 2000 and Ultrazyme 100 were mixed in such a ratio that the final enzyme mixture contained the following activities:

- endo-glucanase (EC 3.2.1.4.): 0.53 Units/ml (on Akzo carboxymethyl cellulose AF 0305).
- exo-glucanase (EC 3.2.1.91): 0.01 Units/ml (on Avicel cellulose, type SF).
- cellobiase (EC 3.2.1.21): 0.09 Units/ml (on Difco cellobiose).
- endo-PG (EC 3.2.1.15): 3.57 Units/ml (on polygalacturonic acid, ICN 102711).
- PE (EC 3.1.1.11): 0.21 Units/ml (on apple green ribbon pectin, DE 62.0%).

Table 15 shows the sugar composition of the tomato WIS after enzymatic prehydrolysis and final hydrolysis with 2 N TFA.

Table 15: Sugar composition (% w/w) of tomato WIS after enzymatic prehydrolysis and 2 N TFA hydrolysis.

| | |
|---|---|
| Rhamnose | 1.1 |
| Arabinose | 1.2 |
| Xylose | 4.2 |
| Mannose | 3.4 |
| Galactose | 2.7 |
| Glucose | 31.9 |

This method of hydrolysis obviously lacks the disadvantages of both the 2 N TFA and the $H_2SO_4$ method of hydrolysis, while the determined glucose content corresponds to the cellulose content as determined according to the method described by Updegraff (1969).

For routine analysis, 3 mg of sample was weighed into a plastic reaction vessel and suspended in 1 ml of enzyme mixture. As control, one reaction vessel with enzyme mixture only was included. The vessels were incubated at 40°C for 48 hours with continous stirring. Subsequently the content was transferred to screw capped tubes and evaporated to dryness under a stream of air at 40°C. The hydrolysis of the sample was completed with 2 N TFA containing myo-inositol as internal standard. Alditol acetates prepared according to Jones and Albersheim (1972) were separated on a gas chromatograph (Hewlett Packard model 5750 G) 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.A.W. 80-100 mesh. The temperature of the injection port was 250°C, of the flame detector 230°C and of the column oven 190°C. The gaschromatograph was connected to a Laboratory Data Control CCM 301 computer which directly calculated the neutral sugar concentration relative to the internal standard. The weight % composition of the neutral sugars was calculated and corrected for the water uptake during the hydrolysis of the polysaccharide. A correction was also made for the neutral sugars present in the enzyme mixture.

*Anhydrogalacturonic (AGA) content and degree of esterification (DE)*

The AGA content and DE of pectin in tomato insoluble solids was determined by the copper ion exchange method described by Keybets & Pilnik (1974) and Katan & v.d. Bovenkamp (1981). Pectin was precipitated as copper pectate and washed to remove the free copper ions. The bound copper ions were released by washing with 0.6 N HCl and determined using an atomic absorption spectrophotometer (Perkin-Elmer, model 2380).
When pectin was degraded to such an extent that no precipitation occurred with copper sulphate (Siliha 1985), the AGA content was determined colourimetrically utilizing m-hydroxydiphenyl as described by Ahmed & Labavitch (1977). Corrections for carbohydrate interference were made according to the method described by Kintner & v Buren (1982).
The concentration of AGA in the fractions collected from gel permeation and ion exchange chromatography was determined by the automated m-hydroxydiphenyl method (Thibault 1979).
The amount of pectin solubilized from WIS as a result of enzyme treatment was determined by the automated m-hydroxydiphenyl method after saponification for 30 min. in 0.05 N NaOH. The DE of this solubilized pectin was calculated from the amount of methanol as determined on a Spectraphysics SP 8000 H.P.L.C. using a 300 x 7.8 mm Aminex HPX 87 H column and a 50 x 4.6 mm AG 50 W-X 4 (Bio-Rad Labs., Richmond, USA) precolumn. Methanol was detected with an ERMA ERC 7510 refractive index detector after elution with 0.6 ml/min 0.01 N $H_2SO_4$ at 30°C. Samples were saponified for 1 hour in 0.05 N NaOH prior to the measurement (Voragen et al. 1986 a).

*Total neutral sugar content*

The total amount of neutral sugars in the eluates of gel permeation chromatography was determined colourimetrically using phenol sulphuric acid as described by Dubois et al. (1956).

*Cellulose content*

The cellulose content in AIS, WIS and their fractions was assessed according to the method described by Updegraff (1969).

*Protein content*

The protein content was determined by the semi-automated

micro-Kjeldahl method described by Roozen & Ouwehand (1978) using a conversion factor of 6.25. Protein solubilized from WIS as a result of enzyme incubation was determined according to the method of Lowry (1951).

*Moisture content*

One g. of sample was transferred to predried, weighed aluminium dishes. The weight loss was determined after 16 hours drying at 106°C.

*Starch content*

The starch content was determined enzymatically using the u.v.-method supplied by Boehringer (Mannheim, FRG).

## 3.4. *Properties of tomato particles*

*Particle size analysis*

Particle size distribution was determined on a Haaver & Boecker laboratory sieve-shaker fitted with sieves of 420, 315, 250, 200, 160, 125, 100 and 56 µm pore size. 200 gram samples of paste were diluted with distilled water to 1 litre and transferred to the sieves. After shaking for 45 min. while rinsing with 4-4.5 litre water, the retained material was washed from the sieves with distilled water. After filtration of these fractions through dried and preweighed filter paper, the filter papers plus solids were dried for 24 hours at 90°C and weighed. The weight of the fractions was recorded as a % of the total amount of insoluble solids present in the sample.

*Microscopic examination of tomato cells*

Tomato juices, concentrates and enzyme treated samples were diluted to approximately 0.5°Bx and examined using an Axiomat Zeiss microscope (phase contrast illumination) equipped with a camera. Photographs were taken on Agfapan 25 film exposed as 18 DIN. The final magnification on the film negative was 51.2 times.

*Degree of sedimentation*

50 gram of a 5°Bx tomato juice or diluted tomato concentrate was weighed in a graduated 100 ml serum bottle together with 100 mg

sodium benzoate. After filling the volume up to 100 ml with distilled water the height of the sedimented particle layer was recorded daily for one week.

*Density gradient centrifugation*

3.5 gram of 5°Bx tomato juice or diluted tomato concentrate was transferred to a centrifuge tube together with 35 gram Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) of density 1.05 (in 0.25 M sucrose). Centrifuging the tubes for 25 min. at 40,000 x g in a Sorvall RC-5B superspeed centrifuge at room temperature resulted in a self-generated density gradient. After centrifugating, the tubes were photographed and layers with different density were isolated and examined microscopically.

## 3.5. *Conductivity measurement*

The conductivity of dialysed tomato juice samples was measured in a WTW model LF 39 conductivity/resistance meter.

## 3.6. *Serum sugars*

Serum sugars were determined on a Spectraphysics SP 8000 H.P.L.C. using a 300 x 7.8 mm Aminex HPX 87 P column and a mixed bed ion exchange precolumn (AG 50 W-X4 ($H^+$form), AG 3-X4A ($OH^-$form), Bio-Rad Labs., Richmond, Cal., USA). Sugars were detected with an ERMA ERC 7510 refractive index detector after elution with 0.5 ml/min. distilled water at 85°C (Voragen et al. 1986c).

## 3.7. *Enzymes: purification and activity determination*

*Endo-polygalacturonase (poly (1,4-α-D-galacturonide) galacturonohydrolase, EC 3.2.1.15)*

Mould PG was isolated from Ultrazyme 20 (Novo Ferment A.G., Basel, Switzerland) and purified as described by Vijayalakshmi et al. (1978). PG activity was assayed colourimetrically 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 a substrate. A mixture consisting of 0.2 ml of 0.2 M sodium succinate buffer pH 5.2, 0.1 ml of polygalacturonic acid solution 1% (w/v) and 0.1 ml 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 of enzyme which catalyses the release of 1 µmol of reducing sugar per minute using glucose as standard.

*Pectinesterase (pectin pectylhydrolase, EC 3.1.1.11)*

Mould PE (Gist-Brocades, Seclin, France) was purified using the method described by Baron et al. (1980). 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 4.0 at 30°C was measured using an automatic titrator (Combi titrator 3D, Metrohm AG, Herisau, Switzerland). The enzyme substrate was a 0.5% (w/v) solution of a commercial apple pectin (Obipectin, green ribbon, DE 62.0%) in 0.15 M NaCl. One unit of PE is the amount of enzyme which liberates 1 µmol of carboxylgroups per minute.

*Endo-pectinlyase (poly(methoxygalacturonide) lyase, EC 4.2.2.10)*

Endo-PL was isolated from Ultrazyme 20 and purified according to the method described by van Houdenhoven (1975). PL activity was determined by monitoring the increase in absorption at 235 nm of a solution of high methoxyl pectin (Voragen 1972). Absorption at 235 nm is specific for the double bonds formed as a result of PL catalysed pectin cleavage. The enzyme substrate consisting of 0.25 ml 1% (w/v) pectin solution (DE = 92.3%), 1.5 ml McIlvaine buffer pH 6.0 and 1.15 ml distilled water, was pipetted into a 10 mm quartz cuvette and placed in a water bath at 30°C. The cuvette was transferred to a temperature controlled chamber in the spectrophotometer and 0.1 ml enzyme solution was added. One unit of PL activity is the amount of enzyme which catalyses the formation of 1 µmol of double bonds per minute. For the calculation of the amount of double bonds, a molar absorption coefficient of 5500 $M^{-1}$ $cm^{-1}$ was used.

*Cellulase complex*

Enzymes forming part of the multi-component cellulase system were isolated

from Maxazym CL, a commercial cellulase preparation from Trichoderma viride origin (Gist Brocades, Delft, The Netherlands), and purified as described by Beldman et al. (1985).

*Exo-glucanase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91)*

The activity was measured in a mixture consisting of 0.5 ml 1% Avicel cellulose (Type S.F., Serva, Heidelberg, FRG) in 50 mM sodium acetate buffer pH 5.0 and 0.5 ml enzyme solution. After incubation for 20 hours at 30°C the mixture was centrifuged. The amount of newly formed reducing endgroups in the clear supernatant was measured using the Nelson-Somogyi method.

*Endo-glucanase (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4)*

The activity was measured in a mixture containing 0.25 ml 1% carboxymethylcellulose (Akucell AF 0305, Akzo, Arnhem, The Netherlands), 0.15 ml 0.1 M sodium acetate buffer pH 5.0 and 0.1 ml enzyme solution. After incubation at 30°C for 1 hr. the reaction was stopped and the reducing sugars were measured by the addition of the Nelson-Somogyi reagent. Samples were centrifuged before reading the absorbance at 520 nm.

*β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21)*

The activity was assayed by the addition of 0.1 ml enzyme solution to 0.9 ml of 0.1% p-nitrophenyl-β-D-glucopyranoside (Koch-Light, Colnbrook, Bucks., England) in 50 mM sodium acetate buffer pH 5.0. After incubation for 1 hr. at 30°C the reaction was stopped by the addition of 1 ml 0.5 M glycinebuffer pH 9.0, containing 2 mM EDTA. The concentration of p-nitrophenol was measured at 400 nm, using an absorption coefficient of 13,700 $M^{-1}cm^{-1}$.

*Cellobiase (a β-glucosidase)*

The activity was determined by the addition of 0.1 ml enzyme solution to 0.1 ml 0.1% cellobiose (Difco, Detroit, Michigan, USA) in $H_2O$, 0.1 ml $H_2O$ and 0.2 ml 0.1 M sodium acetate buffer pH 5.0. After incubation for 20 hours at 30°C the reducing endgroups were measured with the Nelson-Somogyi assay.

*Hemicellulases*

Hemicellulolytic enzymes were isolated from Pectinase no. 29, a commercial preparation from Aspergillus niger origin (Gist-Brocades, Delft, The Netherlands), and purified as described by Rombouts et al. (1986, arabinanases) and Voragen et al. (1986 d, galactanases).

*Arabinofuranosidase (α-L-arabinofuranoside arabinohydrolase, EC 3.2.1.55)*
The activity was measured by the addition of 0.05 ml of enzyme solution to 0.145 ml of 0.1% p-nitrophenyl-α-L-arabinofuranoside (Sigma, St Louis, USA) and 0.35 ml 0.05 M sodium acetate buffer pH 5.0, and incubation for 1 hour at 30°C. The reaction was stopped by the addition of 1 ml 0.5 M glycine buffer pH 9.0, containing 2 mM EDTA. The concentration of p-nitrophenol was measured at 400 nm, using an extinction coefficient of 13,700 $M^{-1}$ $cm^{-1}$.
*Endo-arabinanase (1,5-α-L-arabinan arabinohydrolase, EC 3.2.1.99)*
The activity was measured on apple juice ultrafiltrate, a slightly branched α-1,5-L-arabinan (haze-arabinan, Voragen et al. 1982). The reaction mixture for the endo-arabinanase assay consisted of 0.1 ml 0.5% ultrafiltrate solution in $H_2O$, 0.35 ml 0.05 M sodium acetate buffer pH 5.0 and 0.05 ml enzyme solution. After incubation at 30°C for 1 hour, the reaction was stopped and the reducing sugars were measured by the addition of the Nelson-Somogyi reagent using arabinose as standard.
*Endo-galactanase (1,4-β-D-galactan galactohydrolase, EC 3.2.1.89)*
The activity was determined on a β-1,4-D-galactan isolated from potatoes and in addition treated with arabinofuranosidase in order to remove arabinose side chains from the galactan backbone (Voragen et al. 1986 c). The reaction mixture contained 0.1 ml of 0.5% potato galactan in $H_2O$, 0.35 ml 0.05 M sodium acetate buffer pH 5.0 and 0.05 ml enzyme solution. After incubation at 30°C for 1 hour, the reaction was stopped and the reducing sugars were measured by the addition of the Nelson-Somogyi reagent. Samples were centrifuged before reading absorbance at 520 nm; galactose was used as standard.

# 4. WIS AS A FACTOR DETERMINING GROSS VISCOSITY

## 4.1. Introduction

A survey of relevant literature (chapter 2) shows conflicting opinions regarding the extent to which soluble and insoluble solids contribute to the gross viscosity of tomato products. It is, however, generally accepted that a hot break process yields products higher in gross viscosity than a cold break process. Therefore, in an attempt to clarify the role of soluble and insoluble solids, tomato juice and concentrates prepared according to different break methods were analysed for gross and serum viscosity and chemical composition. Moreover this chapter describes the influence of artificially induced changes in insoluble and soluble solids on the gross viscosity of tomato products.

## 4.2. Methods

### 4.2.1. Preparation of hot break and cold break tomato juice

*Pilot plant*

- Hot break juice. 10 litres of water were heated in a 50 litre scald kettle. 48 kg tomatoes (varieties H30 and H2826) were crushed in a Rossi & Catelli no. 51 chopper at a rate of 1.3 kg/min. and added to the scald kettle. The temperature of the crushed tomatoes in the scald kettle was maintained at 93-98°C. Ten minutes after the last addition to the scald kettle the heated crushed tomatoes were finished using a single stage Rossi & Catelli paddle finisher (1/100/RE) equipped with a 0.6 mm screen. The finished tomato juice was heat processed for 1 hour at 100°C (PHB juice).
- Cold break juice. 20 kg of chopped tomatoes (varieties H30 and H2826) were added to 5 litres of cold water in the scald kettle. The chopped tomatoes were heated to 80°C in 8-10 min. Finishing and heat processing were the same as with the preparation of hot break juice. (PCB juice).

*Laboratory*

- Hot break juice. Tomatoes were quickly heated in a Philips 2010 C commercial microwave oven (type AAH 050, 4.4 kW, 2450 ± 25 MHz) until a heart temperature of 90°C was reached. Heating to this temperature was enough for complete PE and PG inactivation. After cooling to room temperature a correction for evaporation was made by adding distilled water. The tomatoes were finished through a Bauknecht KU2-1 tapered screw juice extractor equipped with a 0.9 mm screen and heat processed for 1 hour at 100°C. (LHB juice).
- Cold break juice. Tomatoes were finished at room temperature using the Bauknecht juice extractor. 15 minutes after the start of finishing the juice was heated in the microwave oven in the same way as with the preparation of hot break juice. The total time for finishing and heating was 30 min. After cooling to room temperature a correction was made for evaporated water. The juice was heat processed for 1 hour at 100°C. ($LCB_1$ juice). Another cold break tomato juice was prepared by heat processing finished tomatoes 5½ hours after the finishing procedure. ($LCB_2$ juice).

*Factory*

- Hot break juice. Hot break tomato juice was prepared in a Rossi & Catelli 1000 L recirculating chop/scalder (Fig. 11).

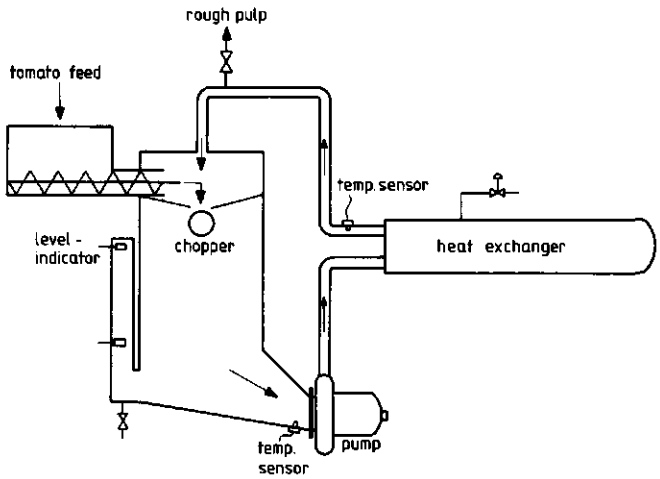



Fig. 11: The Rossi & Catelli 1000L recirculating chop/scalder.

In this system tomatoes are crushed at a rate of 7 tons/hr. and immediately immersed in hot, previously crushed tomatoes. Heating to 103°C is accomplished in the tubular heater section of the chop/scalder. The heated crushed tomatoes were finished using a three stage Rossi & Catelli finisher equipped with 1.0-0.8 and 0.5 mm screens. The juice was heat processed for 1 hour at 100°C. (FHB juice).

- cold break juice. The method of preparing cold break juice was identical to that of hot break juice except for the degree of the break temperature. In the case of the cold break process the break temperature was 40°C instead of 103°C. (FCB juice).

### 4.2.2. *Concentration of tomato juice*

#### *Concentration in an industrial evaporator*

Tomato juice was concentrated in a Rossi & Catelli $T_{30}$ D.F.F. Anteo evaporator. This double effect evaporator is capable of evaporating 12,000 kg water/hr. The fresh tomato juice is received into the second effect of the evaporator which consists of an inclined bank of tubes. The relatively thin tomato juice circulates easily by convection in this section which is heated by the condensing vapours from the first effect. After preconcentration in the second effect the concentrate is transferred by gravity through an automatic level regulator into the first effect of the evaporator. Circulation in this effect is carried out by a turbine which pumps the concentrate down the heating tubes (D.F.F. = Downward Forced Flow). Product temperatures were 60°C in the first effect (58-60 cm Hg vacuum) and 40°C in the second effect (69-70 cm Hg vacuum).

#### *Concentration in a laboratory evaporator*

A Büchi rotary film evaporator was used for the laboratory concentration of tomato juice. Usually the product temperature during concentration was 70°C (52-53 cm Hg vacuum) and the water bath temperature 90°C, however, occasionally the product was concentrated at 20°C (74 cm Hg vacuum) using a water bath temperature of 40°C. The evaporator was equipped with a scraper to prevent burning of the tomato concentrate.

*WIS concentration*

6 tomato juice samples of 250 gram were centrifuged for 45 min. at 25,400 x g in a Sorvall RC-5B refrigerated superspeed centrifuge. After centrifugating 0, 30, 60, 90, 120 and 150 grams of clear serum were removed. After resuspending the particles in the remaining serum, concentrates were obtained with the original serum viscosity but with increased WIS contents.

### 4.2.3. *Reduction of serum viscosity and exchange of serum*

6 x 250 grams of hot break tomato juice of varieties H208 and H318 were centrifuged for 30 min. at 25,400 x g in a Sorvall RC-5B refrigerated superspeed centrifuge. The content of two centrifuge tubes was resuspended and combined (sample code c). To the serum of two other tubes, 44 units purified PG and 51 units purified PE were added. After incubation for 24 hours at 40°C the enzymes were inactivated by heating for 20 min. at 85°C. The enzyme treated serum was resuspended with the residue in the centrifuge tubes and the content of the two tubes was combined (sample code e). The serum of the third set of centrifuge tubes was combined and exchanged with the corresponding serum from juice of the other variety. Serum of one variety was resuspended with residue of the other variety (sample code s).

### 4.2.4. *Dialysis of tomato juice*

2 kg of tomato juice was dialysed, in bags of cellulose acetate, for 3 days at 4°C against 6 volumes of 25 litres distilled water. The dialysed tomato juice was concentrated and the viscosity of the concentrates was compared to non-dialysed concentrates.

## 4.3. *Results and Discussion*

### *Pilot plant hot and cold break*

In the 1980 season pilot plant hot and cold break tomato juice was prepared from tomato varieties H30 and H2826 as described under 4.2.1. The tomatoes were roundish, fully ripe, and had a mean weight of 99 gram and 66 gram for variety H30 and H2826 respectively. The juice was concentrated using the Büchi laboratory rotary film evaporator.

Figures 12 and 13 show gross and serum viscosities of the juices and their concentrates as related to the soluble solids content (°Bx). It is clear that there were large varietal differences . At a specified soluble solids level a variety H2826 concentrate has almost double the gross viscosity of a H30 variety concentrate (Fig. 12).

In contrast to what is generally assumed, no difference in gross viscosity was found between hot break and cold break juices and concentrates in spite of large differences in serum viscosity (Fig.13).

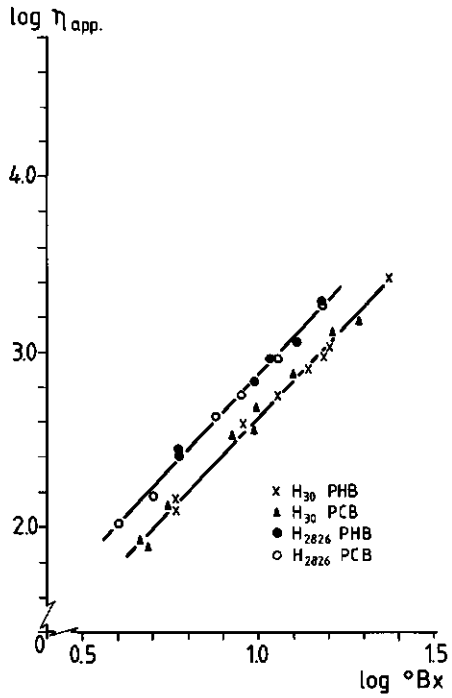



Fig. 12: Relationship between °Bx and gross viscosity for hot break juice, cold break juice and their concentrates (varieties H30, H2826).

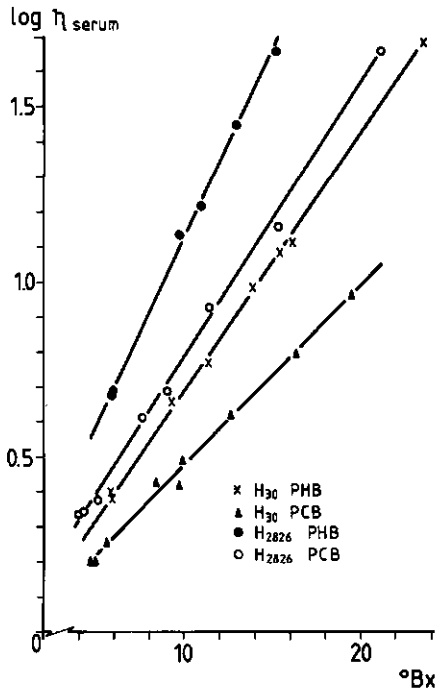



Fig. 13: Relationship between °Bx and serum viscosity for hot break juice, cold break juice and their concentrates (varieties H30, H2826).

Table 16: Solids content of tomato juice prepared from varieties H30 and H2826.

| sample | °Bx | TS % w/w | AIS % w/w | s-AIS % w/w | WIS % w/w |
|---|---|---|---|---|---|
| H30 PHB | 5.8 | 6.38 | 1.03 | 0.25 | 0.89 |
| H30 PCB* | 4.6 | 4.84 | 0.71 | 0.10 | 0.65 |
| H2826 PHB | 5.9 | 6.75 | 1.52 | 0.40 | 1.26 |
| H2826 PCB* | 4.0 | 4.60 | 0.96 | 0.19 | 0.76 |

* low °Bx due to addition of water before break (see 4.2.1.).

Table 16 shows that the main effect of the cold break is a decrease in s-AIS amount, even when compared at identical °Bx; this is in accordance with the low serum viscosities found. The low s-AIS values are caused by the degradation of large parts of the high molecular weight serum soluble pectin into lower molecular weight fragments not precipatable with alcohol (Table 17).

Table 17: AGA content of AIS, s-AIS and WIS prepared from hot and cold break juice of two varieties (weight % on basis of fresh weight x 100 at 5°Bx).

| | H30 | | H2826 | |
|---|---|---|---|---|
| | PHB | PCB | PHB | PCB |
| AIS | 21.3 | 16.2 | 33.3 | 24.8 |
| s-AIS | 12.8 | 6.2 | 18.2 | 12.1 |
| WIS | 7.8 | 7.9 | 11.9 | 9.9 |

Tables 16 and 17 also indicate minor changes occurring to the WIS; the WIS pectin is affected as is illustrated by the decrease in overall degree of esterification (Table 18).

Table 18: Overall DE of the pectin in AIS and WIS fractions of juice from varieties H30 and H2826.

| sample | AIS | s-AIS | WIS |
|---|---|---|---|
| H30 PHB | 55 | 65 | 35 |
| H30 PCB | 46 | 66 | 21 |
| H2826 PHB | 59 | 69 | 40 |
| H2826 PCB | 56 | 66 | 29 |

Table 19: Sugar composition of AIS, WIS and s-AIS prepared from tomato juice of two varieties (Mol%).

| | AIS | | WIS | | s-AIS | |
|---|---|---|---|---|---|---|
| | PHB | PCB | PHB | PCB | PHB | PCB |
| H30 | | | | | | |
| Rhamnose | 0.7 | 0.7 | 0.3 | 0.3 | 1.1 | 1.0 |
| Arabinose | 2.7 | 2.9 | 4.9 | 4.5 | 1.6 | 3.1 |
| Xylose | 7.3 | 8.3 | 6.8 | 6.7 | 1.2 | 1.5 |
| Mannose | 4.5 | 5.3 | 11.1 | 9.3 | 1.0 | 0.6 |
| Galactose | 4.3 | 6.3 | 5.1 | 6.2 | 3.1 | 7.5 |
| Glucose | 33.0 | 36.1 | 47.2 | 45.0 | 6.7 | 5.8 |
| AGA | 47.6 | 40.5 | 24.5 | 28.1 | 85.5 | 80.7 |
| H2826 | | | | | | |
| Rhamnose | 0.5 | 0.6 | 0.3 | 0.3 | 0.6 | 0.6 |
| Arabinose | 5.0 | 5.7 | 5.5 | 5.8 | 5.5 | 7.6 |
| Xylose | 7.2 | 7.8 | 8.5 | 8.0 | 1.4 | 1.1 |
| Mannose | 4.3 | 5.3 | 8.1 | 9.6 | 1.2 | 0.8 |
| Galactose | 5.7 | 8.2 | 6.3 | 7.7 | 5.7 | 12.7 |
| Glucose | 31.7 | 34.8 | 45.7 | 46.7 | 7.5 | 5.2 |
| AGA | 45.7 | 37.7 | 25.6 | 21.8 | 78.3 | 72.2 |

Although large parts of the serum pectin are degraded during cold break as a result of pectolytic enzyme action, the overall DE of the remaining high molecular weight serum pectin is almost unchanged. An explanation for this observation can probably be found in Table 19, which shows the sugar composition of the hot and cold break samples. The molar amounts of arabinose and galactose in the s-AIS of cold break juice have increased greatly when compared to hot break juice. This may indicate that during cold break, pectolytic enzymes initially attack an easily accessible soluble pectin, e.g. a pectin without side chains. After saponification and degradation of this pectin, the remaining serum pectin is still highly esterified, probably because this pectin is protected against enzymatic attack by side chains of arabinose and galactose.

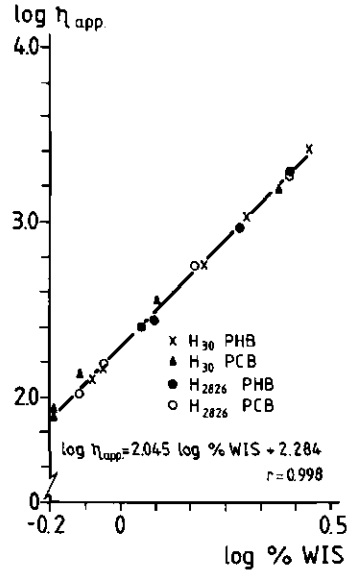



Fig. 14: Relationship between %WIS and gross viscosity for hot break juice, cold break juice and their concentrates (varieties H30, H2826).

The data show that degradation of serum pectin and the resulting decrease in serum viscosity does not necessarily affect gross viscosity. In the case of the pilot plant cold break, the samples were heated to a temperature of 80°C within 8-10 min. after crushing. Therefore pectolytic enzyme action was probably restricted mainly to the easily accessible pectin in the serum while the insoluble solids were more or less unaffected. Altogether, the

results point to the importance of the water insoluble solids in determining gross viscosity. Moreover, this is ilustrated in Fig. 14, which shows gross viscosity for hot and cold break samples of both varieties as a function of the WIS content. Obviously the varietal difference in gross viscosity as observed in Fig. 12 is only caused by a difference in WIS content (Table 16).

*Laboratory hot and cold break*

Class I glasshouse tomatoes of the variety Sonatine were obtained from the Sprenger Institute, Wageningen, the Netherlands on 11/6/1981 and immediately stored at 3°C in the dark. The tomatoes were fully ripe and had a mean weight of 66 g/tomato. Hot and cold break juice samples were prepared both after 4 days and 3 weeks storage at 3°C. Storage caused a 5% decrease in AIS content of the tomatoes; however, the AIS composition did not change.

Table 20: Solids content of juice prepared from tomato variety Sonatine after 4 days and 3 weeks storage at 3°C.

| Sample | °Bx | TS % w/w | AIS % w/w | s-AIS % w/w | WIS % w/w |
|---|---|---|---|---|---|
| $LCB_1$ 4 days | 5.0 | 5.71 | 0.95 | 0.32 | 0.80 |
| $LCB_2$ 4 days | 5.2 | 5.90 | 0.96 | 0.21 | 0.80 |
| $LCB_1$ 3 weeks | 5.0 | 5.58 | 0.91 | 0.19 | 0.72 |
| $LHB_1$ 3 weeks | 5.1 | 5.88 | 1.09 | 0.35 | 0.74 |

During the preparation of pilot plant cold break juice, pectolytic enzymes acted maximally 10 min. on the coarse crushed tomatoes before they were heat inactivated. During the laboratory preparation of cold break juice, however, the enzymes were active on the finished product (0.9 mm) for at least 15 min. and for as long as 5½ hours before they were heat inactivated. Even the long enzyme action did not result in detectable water

insoluble solids breakdown (Table 20), possibly because the incubation temperature of ± 20°C was unfavourable for high enzyme activity. As with the pilot plant samples, the laboratory hot and cold break samples did not differ in gross viscosity in spite of very large differences in serum viscosity (Fig. 15 and 16).

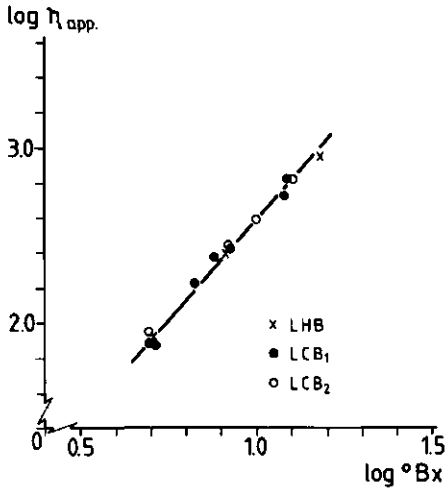



Fig. 15: Relationship between °Bx and gross viscosity for hot break juice, cold break juice and their concentrates (variety Sonatine).

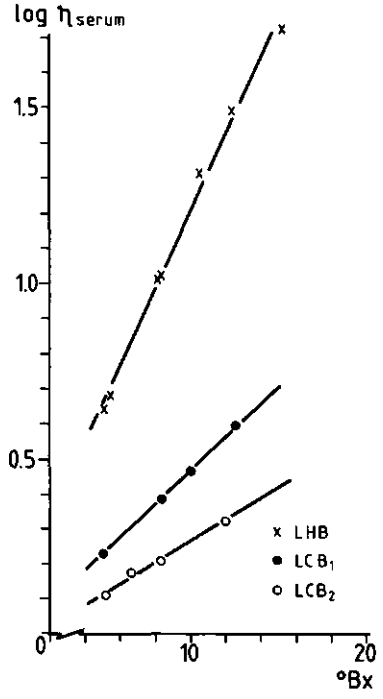



Fig. 16: Relationship between °Bx and serum viscosity for hot break juice, cold break juice and their concentrates (variety Sonatine).

Table 21: AGA content of AIS, s-AIS and WIS prepared from tomato juice of variety Sonatine (weight % on basis of fresh weight x 100 at 5°Bx). In parentheses the degree of esterification of the AGA

| | LHB | $LCB_1$ | $LCB_2$ |
|---|---|---|---|
| AIS | 24.2 (65) | 17.6 (35) | 14.9 (14) |
| s-AIS | 20.3 (69) | 9.6 (45) | - |
| WIS | 4.9 (37) | 6.4 (6) | - |

Pectin degradation by enzyme action was again restricted to the soluble serum pectin. However, in case of the laboratory cold break juice, the enzymes acted long enough to partly saponify the remaining undegraded serum pectin (Table 21) and to degrade high molecular weight polymers containing 50% of the serum arabinose and 30% of the serum galactose to alcohol soluble fragments (data not shown). The longer action of the pectolytic enzymes is also indicated by the very low overall degree of esterification of the WIS-pectin. From the data in Table 21 it appears that a part of the saponified, undegraded serum pectin has precipitated in the WIS. Leoni et al. (1979, 1980, 1981) also reported precipitation of saponified serum pectin as a result of a cold break treatment.

*Factory hot and cold break*

In the 1981 season, hot and cold break juices were prepared on factory scale from the tomato variety H1361. The tomatoes were mature, red coloured and had a mean weight of 122 g/tomato. The Rossi & Catelli T30 evaporator was used to concentrate the juice to several solids levels.

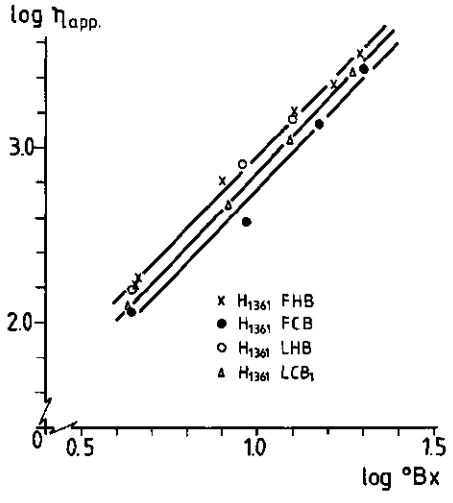



Fig. 17: Relationship between °Bx and gross viscosity for hot break juice, cold break juice and their concentrates (variety H1361).

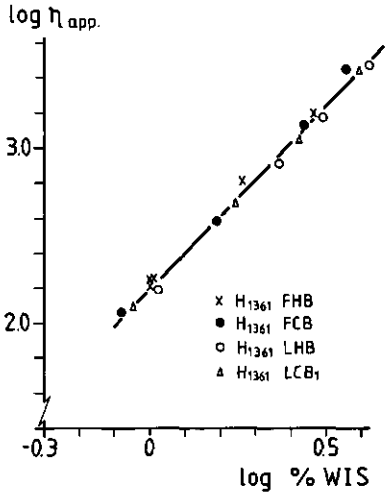



Fig. 18: Relationship between %WIS and gross viscosity for hot break juice, cold break juice and their concentrates (variety H1361).

Laboratory hot and cold break juices were prepared from the same variety as a reference. These juices were concentrated with the rotary film evaporator.

Fig. 17 shows the gross viscosity of the juice and concentrates as related to the soluble solids (°Bx). No difference in gross viscosity can be detected between the two hot break samples; however, the cold break samples show a distinctly lower gross viscosity. The gross viscosity of the factory cold break sample is approximately 60% of the factory hot break sample. This difference is obviously caused by the lower amount of WIS in the cold break juice (Table 22). The enzyme action during the factory cold break resulted in a complete degradation of serum pectin and in saponification of WIS pectin. However, the results do not give a decisive answer to the question whether the WIS was also degraded by the enzymes. It is certain that the composition of hot break juice WIS differs from cold break juice WIS, but it cannot be excluded that this difference and the difference in WIS amount could be a result of processing conditions (e.g. finishing operation, see 7.3.2.) other than break temperature.

Table 22: Solids content, serum viscosity and composition of factory and laboratory tomato juices of variety H1361.

| sample | °Bx | %TS | %WIS | $\eta_{serum}$ at 20°Bx | Composition of WIS* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | AGA | DE | Protein | Cellulose |
| FHB | 4.6 | 5.34 | 1.05 | 71 | 14.5 | 23 | 21.6 | 40.7 |
| FCB | 4.4 | 5.02 | 0.84 | 2 | 10.2 | 0 | 17.6 | 36.9 |
| LHB | 4.4 | 5.20 | 1.07 | 188 | - | - | - | - |
| $LCB_1$ | 4.3 | 5.07 | 0.90 | 4 | - | - | - | - |

* expressed as weight % on basis of fresh weight x 100 at 5°Bx.

Figure 18 shows that all the hot and cold break samples prepared from variety H1361 have equal gross viscosities at a specific WIS amount in spite of the differences in WIS amount and WIS composition.

Figure 19 shows microscopic pictures of industrially prepared Greek hot and cold break pastes of unknown variety. From these pictures it is clear that the WIS in this cold break paste has been degraded.

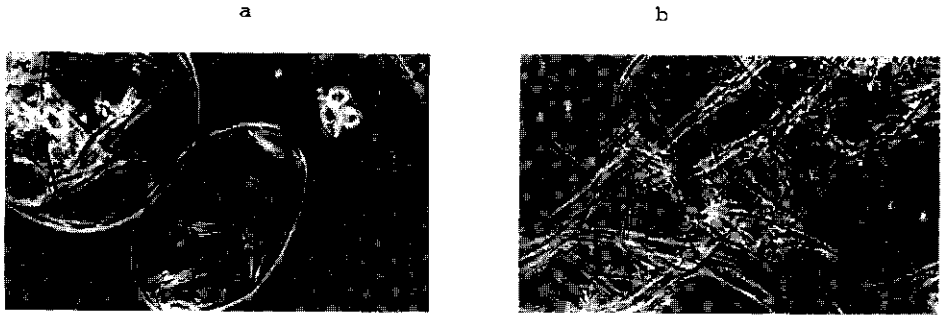



Fig. 19: Tomato cells as present in hot and cold break paste. a: hot break paste, b: cold break paste (magnification 61.4x).

Particle size analysis (Fig. 20) also clearly shows the degradation of the tomato cells.

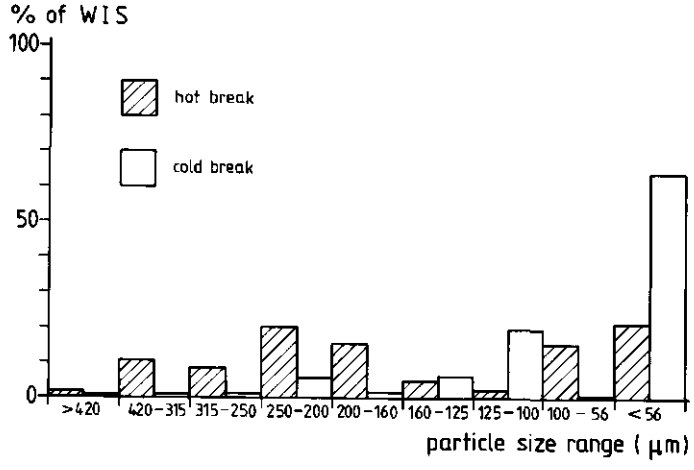



Fig. 20: Difference in size of particles present in hot break and cold break paste.

Table 23: Solids content and viscosities of diluted hot and cold break Greek tomato paste.

| | °Bx | %TS | %WIS | %s-AIS | %AIS | $\eta_{app}$ | $\eta_{serum}$ |
|---|---|---|---|---|---|---|---|
| hot break | 9.1 | 10.55 | 1.57 | 0.38 | 1.87 | 230 | 6.4 |
| cold break | 9.2 | 10.15 | 1.30 | 0.20 | 1.40 | 136 | 1.3 |

This cold break paste contains 18% less WIS than the hot break paste (Table 23). Serum pectin has been almost completely degraded as is indicated by the low serum viscosity. Analysis of the neutral sugar composition of the WIS confirms the degradation of the WIS pectin (Table 24).
68% of the WIS pectin has been solubilized together with 40% of the WIS arabinose and 30% of the WIS galactose. While the WIS pectin is not only solubilized but also degraded, the solubilized arabinose and galactose remain as high molecular weight polymers and precipitate in the s-AIS. This, together with the degradation of 72% of the serum pectin, causes the large increase in molar amounts of arabinose and galactose in the s-AIS as a result of the cold break.

Table 24: Sugar composition (Mol%) of Greek hot and cold break tomato paste.

| | WIS | | s-AIS | |
|---|---|---|---|---|
| | hot break | cold break | hot break | cold break |
| Rhamnose | 0.2 | - | 0.4 | 3.3 |
| Arabinose | 5.5 | 4.4 | 3.1 | 14.7 |
| Xylose | 13.0 | 16.4 | 0.9 | 4.6 |
| Mannose | 6.3 | 8.4 | 0.6 | 0.1 |
| Galactose | 7.3 | 6.9 | 3.8 | 20.7 |
| Glucose | 39.0 | 52.0 | 5.0 | 6.0 |
| AGA | 28.7 | 12.1 | 86.2 | 50.5 |

The pectin in WIS and serum has been degraded to such an extent that determination of the AGA content with the copper precipitation method was no longer possible, therefore the reported AGA contents were determined with the m-hydroxydiphenyl method as described by Ahmed & Labavitch (1977). The degradation of the WIS not only resulted in a lower gross viscosity at a specific °Bx value but also in a lower gross viscosity at a specific WIS amount. The WIS of this cold break sample is apparently of poorer quality than that of the hot break sample. (Fig. 21 and 22).

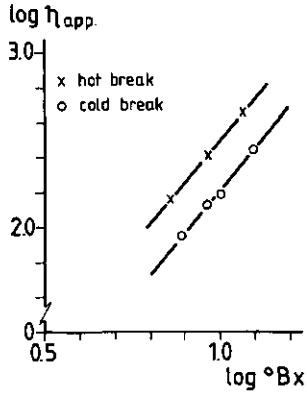



Fig. 21: Relationship between °Bx and gross viscosity for hot break paste and cold break paste produced in Greece.

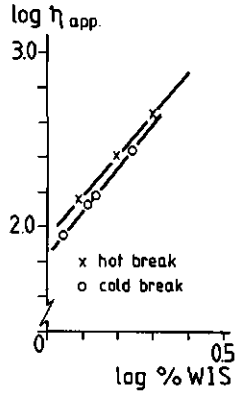



Fig. 22: Relationship between %WIS and gross viscosity for hot break paste and cold break paste produced in Greece.

*WIS concentrates*

The use of the WIS concentration technique in studying the influence of soluble and insoluble solids on gross viscosity offers the opportunity to prepare samples with identical serum viscosity and amount of soluble solids but with increasing amounts of WIS of uniform quality. Comparison of gross viscosity of such concentrates with normal rotary film evaporator concentrates will show the influence of WIS content and serum viscosity on gross viscosity.

From the results, presented graphically in Fig. 23, it can be concluded that no difference exists in gross viscosity of WIS or evaporator concentrates in spite of a large difference in serum viscosity. Serum viscosity again was not found to play a detectable role in the determination of gross viscosity.

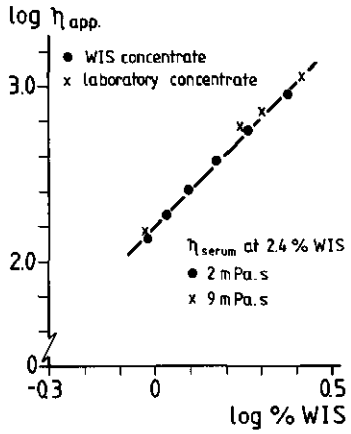



Fig. 23: Relationship between %WIS and gross viscosity for concentrates prepared according to two methods.

## *Degradation of serum pectin and exchange of serum*

Table 25: Effect of a change in serum viscosity on gross viscosity of tomato juice and concentrate.

| tomato variety | | juice | | | | | concentrate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | code[1] | °Bx | %WIS | $\eta_{serum}$ | $\eta_{app}$ | Bostwick | °Bx | %WIS | $\eta_{serum}$ | $\eta_{app}$ | Bostwick |
| 208 | n.c | 4.6 | 1.22 | - | 239.3 | 9.8 | 9.0 | 2.45 | - | 885.2 | 2.4 |
| | c | 4.6 | 1.15 | 2.9 | 203.2 | 10.9 | 9.8 | 2.79 | 9.6 | 1040.6 | 1.6 |
| | e | 4.6 | 1.17 | 1.3 | 216.4 | 9.5 | 11.0 | 3.35 | 2.2 | 1278.5[3] | <1 |
| | s | 4.8 | 1.20 | - | 224.8 | 9.8 | 10.7 | 2.69 | - | 1022.7 | 1.6 |
| 318 | n.c | 4.9 | 1.02 | - | 174.9 | 11.9 | 10.4 | 2.11 | - | 678.2 | 3.5 |
| | c | 5.0 | 0.99 | 2.7 | 161.7 | 12.2 | 10.6 | 2.19 | 8.3 | 721.1 | 3.7 |
| | e | 4.9 | 1.00 | 1.2 | 175.7 | 11.4 | 10.8 | 2.24 | 2.0 | 714.6 | 3.5 |
| | s | 4.6 | 0.99 | - | 165.1 | 12.1 | 10.6 | 2.35 | 10.9 | 820.6 | 3.1 |

1) code: n.c = not centrifuged, c = centrifuged, e = enzyme treated, s = serum exchange

2) $\eta_{serum}$ at 10°Bx: 208 = 10.1 mPa.s, 318 = 7.4 mPa.s

3) too low as result of separation during Haake measurement.

Table 25 shows the results of an experiment in which the serum pectin is degraded by the combined action of purified PE and PG without any change in WIS or other soluble solids. Another possibility of changing the serum viscosity in a tomato juice is to exchange its serum for the serum of another tomato juice, results of this experiment are also included in Table 25. A logaritmic relationship was again found between the gross viscosity (both Haake and Bostwick viscosity) and the WIS content of the sample, independently of the serum viscosity. These results can lead to no other conclusion than that the gross viscosity of a tomato juice or concentrate is mainly determined by its WIS content.

*Dialysis of tomato juice*

The dialysis of tomato juice results in the removal of low molecular weight sugars such as glucose, fructose and saccharose, and in the removal of electrolytes (Tables 26 and 27). Dialysis does not change the WIS and serum pectin content of a juice. Whittenberger & Nutting (1958) and Foda & McCollum (1970) assumed that the electrolytes have a negative influence on gross viscosity.
63% of the measured °Bx value is determined by the neutral sugars glucose, fructose and saccharose (Table 26).

Table 26: Influence of dialysis on composition of tomato juice.

| | °Bx | %TS | %WIS | serum sugars mg/ml. | | |
|---|---|---|---|---|---|---|
| | | | | glucose | fructose | saccharose |
| not dialysed | 5.5 | 6.25 | 0.94 | 15.92 | 17.32 | 1.26 |
| dialysed | 1.0 | 2.17 | 0.94 | 2.92 | 3.02 | 0.37 |

82% of these sugars have been removed during dialysis, corresponding to 68% of the decrease in total solids. This means that 1.3 gram/100 g juice of other substances have been removed during dialysis. Table 27 shows the effect of this removal on pH and conductivity.

Table 27: Effect of dialysis on pH and conductivity.

| | °Bx | %TS | %WIS | pH | conductivity (μmho) |
|---|---|---|---|---|---|
| not dialysed | 5.6 | 6.29 | 0.94 | 4.33 | $7.49 \times 10^3$ |
| dialysed + sugar added | 5.1 | 5.13 | 0.92 | 4.05 | $7.41 \times 10^2$ |

The influence of dialysis on gross viscosity, as measured with the Bostwick consistometer, and on serum viscosity is shown in Table 28. Gross viscosity of dialysed tomato juice measured with the Haake rotovisco did not differ from gross viscosity of juice which was not dialysed.

Table 28: Influence of dialysis on gross viscosity and serum viscosity

| %WIS | not dialysed | | dialysed | |
|---|---|---|---|---|
| | Bostwick | $\eta_{serum}$ | Bostwick | $\eta_{serum}$ |
| 1.0 | 12.5 | 2.6 | 13.4 | 2.5 |
| 1.5 | 6.5 | 4.4 | 6.9 | 3.8 |
| 2.0 | 3.4 | 7.7 | 3.5 | 5.7 |
| 2.5 | 1.7 | 13.2 | 1.8 | 8.7 |

However, due to separation in the measuring gap the use of the Haake rotovisco for gross viscosity measurements on concentrates of dialysed juice was not possible. Table 28 does not indicate an influence of the dialysed sugars and electrolytes on gross viscosity. The removal of these components decreased serum viscosity, but again the decrease in serum viscosity did not affect gross viscosity.

## 4.4. *Conclusions*

Figures 24 to 26 present a summary of gross and serum viscosity data determined during the seasons 1980-1984 on tomato juice and concentrates

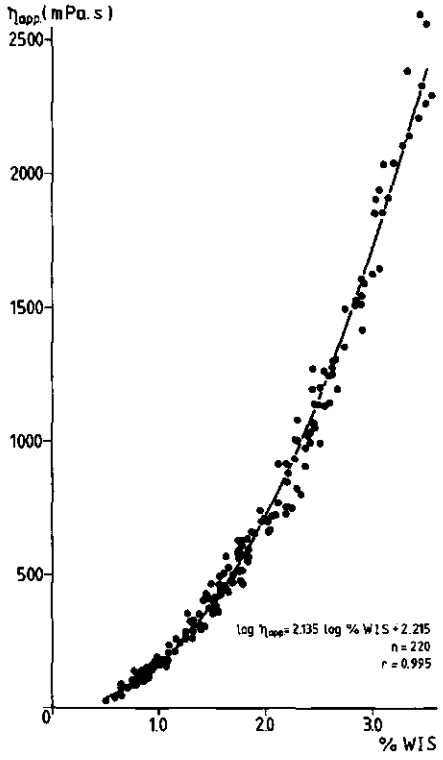



Fig. 24: Relationship between WIS content and gross viscosity as determined with the Haake rotovisco.

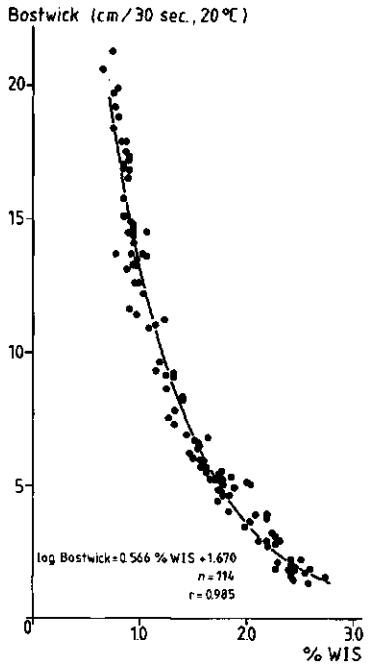



Fig. 25: Relationship between WIS content and gross viscosity as determined with the Bostwick consistometer.

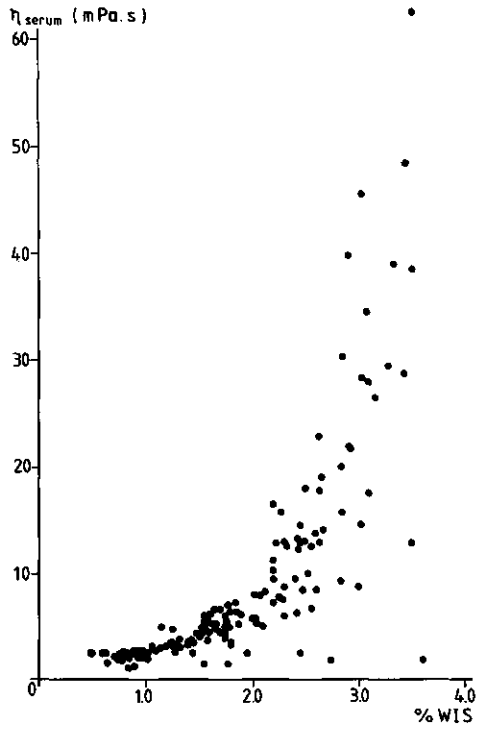



Fig. 26: Relationship between WIS content and serum viscosity for the same samples as shown in Figures 23 and 24.

from varieties H30, H1361 and H2826. Apart from a difference in variety, the analysed samples also differed in methods of production and concentration. Figures 24 and 25 show that in spite of all these differences there is a strong relationship between Haake or Bostwick gross viscosity and WIS contents. Figure 26, on the contrary, shows a very large spread in serum viscosity data at these WIS contents.

The results of the experiments discussed in this chapter prove that the gross viscosity of tomato juices and concentrates is determined primarily by their WIS content. Serum viscosity, which is strongly influenced by break temperature, has no effect on gross viscosity. Variety and processing method influence gross viscosity at a specific °Bx by affecting the amount of WIS. As will be discussed in chapter 7, the processing method can also affect the quality of the WIS.

# 5. COMPOSITION AND STRUCTURAL FEATURES OF TOMATO WIS

## 5.1. Introduction

The elucidation of the chemical structure of plant cell walls has been the subject of many studies. At this time, however, little can be said about the secondary and tertiary structure of the cell wall polymers (McNeil et al. 1984, Dey & Brinson 1984). The structure of the cell wall is obviously much more complex than Keegstra et al. (1973), Robinson (1977) or Lamport & Epstein (1983) have assumed in their models.

From the available literature (section 2.3.2.) it can be concluded that the firmness of tomato fruit is strongly influenced by the cell wall polysaccharides (WIS) and their chemical and biochemical changes during ripening. Results presented in the preceding chapter stress the importance of the WIS in determining the rheological properties of tomato products.

The aim of the experiments described in this chapter was to obtain more knowledge on both the composition of the tomato cell wall and the structural features of tomato cell wall polysaccharides. WIS isolated from fresh tomatoes and tomato products was fractionated either by use of solvents like ammonium oxalate, HCl and NaOH, or by use of purified enzymes. The fractions obtained were further characterized by gel permeation and ion exchange chromatography, and by analysis of neutral sugar and glycosidic linkage composition.

## 5.2. Methods

### 5.2.1. Fractionation of tomato WIS

The WIS of fresh tomatoes, tomato juice and tomato paste was fractionated into pectin, hemicellulose and cellulose fractions as shown in Fig. 27a. In some instances the pectic substances were fractionated according to their solubility in 0.2% ammonium oxalate, 0.05 N HCl and 0.05 N NaOH + 5 mM EDTA (Fig. 27b).

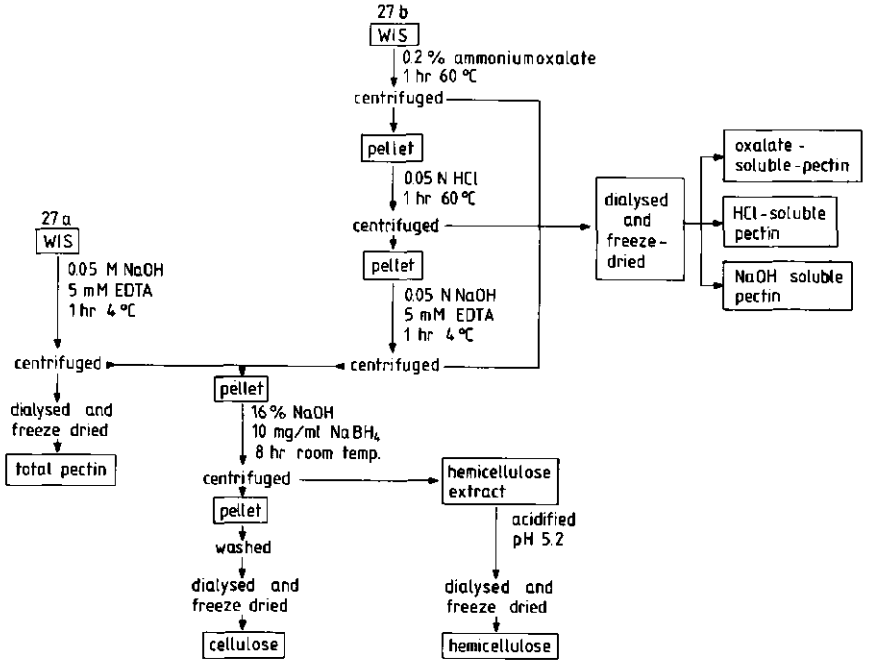



Fig. 27: Extraction of polysaccaride fractions of tomato WIS.

Each fractionation was carried out by extracting three times, under continuous stirring, with the extractant (100 ml/g WIS) and twice with distilled water. After each extraction, the mixture was centrifuged in a Sorvall RC-5B refrigerated superspeed centrifuge for 20 min. at 25,400 x g (4°C). The combined supernatants were dialysed against distilled water for 5 days and freeze-dried. The neutral sugar composition and uronide content of the fractions were determined.

### *5.2.2. Characterization of pectin fractions*

10 mg quantities of ammonium oxalate soluble and HCl soluble pectin were dissolved in 0.05 M sodium acetate buffer pH 6.0. In the case of ammonium oxalate soluble pectin a small amount of remaining insoluble material was removed by centrifugation. The clear supernatant was applied to a 60 x 1.2 cm column of Sephacryl S-500 (Pharmacia Fine Chemicals, Uppsala, Sweden; fractionation range 40,000 - 2 x $10^7$ measured on dextran)

and eluted with 0.05 M sodium acetate buffer pH 6.0 at a flow rate of 15 ml/hr. and at ambient temperature. Fractions of 2 ml were collected and assayed for uronides and total neutral sugar content. The molecular weight of fragments separated using gel permeation chromatography was estimated, using dextrans as a reference and reported as apparent molecular weight. On one occasion, fractions obtained by chromatography of HCl soluble pectin were also analysed for neutral sugar composition.
Fractions containing the lower molecular weight fragments were pooled, concentrated and again applied to a Sephacryl S-500 column. This time, however, the column was eluted with distilled water. The separation achieved upon elution with water is not based solely on size exclusion; uronide containing fractions are excluded from the column matrix, presumably due to charge effects, and therefore elute in the void of the column. The ammonium oxalate soluble pectin was also fractionated on a 60 x 1.2 cm column of Sephacryl S-200 (fractionation range 1000-80,000 measured on dextran). Eluent, flow rate, fraction size and analyses of the fractions were the same as for fractionation of this pectin on Sephacryl S-500.

### *5.2.3. Characterization of the hemicellulose fraction*

Ion exchange chromatography: 150 mg quantities of hemicellulose were dissolved in 20 ml distilled water by stirring for 24 hr. at 20°C. The insoluble material was removed by centrifugation. The clear supernatant was applied to a 15.0 x 2.2 cm column of DEAE-cellulose in acetate form (Whatman DE 52) equilibrated with distilled water. 10 ml fractions were eluted from the column (100 ml/hr.) with a stepwise gradient of successively 120 ml distilled water; 100 ml portions of potassium acetate buffer pH 7.0, respectively 0.05 M, 0.1 M, and 0.5 M; 100 ml sodium hydroxide solution 0.1 M, 80 ml sodium hydroxide 0.2 M, 110 ml sodium hydroxide 0.5 M and finally with 290 ml sodium hydroxide 1 M to wash the column. The experiments were performed at ambient temperature. Sugar containing fractions forming a peak were pooled, dialysed and freeze-dried. From the total sub-fraction the sugar composition and glycosidic linkage composition were estimated. Glycosidic linkage composition was established by permethylation according to the method described by Hakomori (1964)

followed by 2 N trifluoroacetic acid hydrolysis as described by Talmadge et al. (1973). The sugar derivatives were identified and determined by capillary-glc and by combined glc-ms. For capillary-glc a 10 m x 0.32 mm, wall coated OV-225 column in a Carlo-Erba Fractovap 4160 gas chromatograph was used. Samples were injected cold-on-column, the oven temperature was held for 1 min. at 150°C, then raised to 200°C with a rate of 2°C/min. and held at 200°C for 2 min. Peaks were recorded and integrated with a LDC 301 computing integrator. Glc-ms was performed on a V.G.-MM 7070 F mass spectrometer coupled to a Pye 204 gas chromatograph equipped with a 1.5 m column packed with OV-225 (3%) on Chromosorb WHP.
To enable differentiation between 1,2 and 1,4 linked xylose residues, reduction to alditols was also carried out with sodium borodeuteride.

Gel permeation chromatography: 5-10 mg of hemicellulose in 0.5 ml distilled water (clarified by centrifugation) was applied to a 60.0 x 1.2 cm column of Sephacryl S-500 and eluted with distilled water at a flow rate of 10 ml/hr. Fractions of 2 ml were collected and assayed for uronides and neutral sugars. Sugar containing fractions were analysed for sugar composition.
To 0.5 ml of a clear hemicellulose solution (5-10 mg in 0.01 M sodium acetate buffer pH 5) was added 0.3 units of a purified 1,4-β-D-glucan-glucanohydrolase (E.C. 3.2.1.4) isolated from a technical cellulase preparation (Maxazym CL 2000, Gist-Brocades, Delft, The Netherlands). The reaction mixture was incubated for 20 hrs. and fractionated by gel permeation chromatography on Sephacryl S-500. The included fraction which contained the major part of the neutral sugars was further chromatographed over a 100 x 2.6 cm column of Bio-Gel P-2 (Bio-Rad, Richmond, Cal., USA; fractionation range 100-1800 measured on globular biomolecules) thermostated at 50°C and eluted with distilled water at a flow rate of 25 ml/hr. Fractions of 2.5 ml were collected and assayed for total neutral sugars. The sugar composition of pooled fractions was established.

### 5.2.4. *Enzymatic fragmentation of WIS and characterization of the fragments solubilized*

Enzyme incubations: 50-150 mg quantities of WIS were suspended in 5-10 ml 0.05 M sodium acetate buffer pH 5.0. Enzymes were added (Table 29) and the mixture was incubated for 48 hours at 37°C. After the incubation, the solubilized fragments were separated from the residue by means of centrifugation and filtration. The residues were washed twice with distilled water; washing water and reaction liquid were combined and made up to 10 or 25 ml with distilled water. The solubilized fragments were assayed for neutral sugar composition, uronide and protein content.
The residues were resuspended in distilled water and freeze-dried. Some of these residues were treated further with other types of enzymes.

Gel permeation chromatography: Reaction liquids from the control (WIS incubated with 0.05 M. sodium acetate buffer pH 5.0) and for PL, PG, and galactanase treated WIS, were applied to a 60 x 1.2 cm column of Sephacryl S-200 and eluted with 0.05 M sodium acetate buffer pH 6.0. Fractions of 2 ml were collected and assayed for total neutral sugar and uronide content. Fractions forming a peak were pooled and analysed for neutral sugar composition. Depending on the fractionation pattern the pools were further fractionated on a 60 x 1.2 cm column of Sephacryl S-200 (water as eluent), Sephacryl S-500 (0.05 M sodium acetate buffer pH 6.0 as eluent) or on a 90 x 1.0 cm column of Bio-Gel P-2 (water as eluent). 1 ml fractions were collected in the case of fractionation over Bio-Gel P-2; in the other cases 2 ml fractions were collected. The fractions were analysed for total neutral sugars and uronide content; pooled peaks were assayed for neutral sugar composition.
The reaction liquid obtained by incubation of WIS with endo-glucanase + exo-glucanase was fractionated on Sephacryl S-500. The included peak of this separation was further fractionated on Bio-Gel P-2. All the columns were eluted with water; the fractions were analysed for total neutral sugar and uronide content, the pooled peaks for neutral sugar composition.
Some of the reaction liquids obtained after the incubation of residues of enzyme treated WIS with new enzymes, were also fractionated on Sephacryl S-200. The fractions and peaks were analysed as mentioned above.

Table 29: Activity and purity of enzymes used for solubilization of fragments from WIS.

| Enzyme | Activity U/ml | Protein mg/ml | ml enzyme to 50 mg WIS | activities found in enzymes* | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | xylan (β-1,4) | arabinan (α-1,5, α-1,3) | haze arabinan (α-1,5) | arabinogalactan (β-1,3, β-1,6) | potato galactan (β-1,4) | pentosan (arabinoxylan) | gentobiose (β-1,6 glucose) | postulan (β-1,6 glucose) | pachyman (β-1,3 glucose) | laminarin (β-1,3, β-1,6 glucose | pnp-β-D-glucoside | carboxymethylcellulose | avicell | polygalacturonic acid | pectin DE 92 (PL) | pectin DE 62 (PE) | brown ribbon pectin |
| PG | 19.1 | 0.05 | 0.25 | ± | - | - | | - | | | | | | | | | ++ | - | - | |
| PE | 64.0 | 0.25 | 0.11 | | | | | | | | | | | | | | - | - | ++ | |
| PL | 24.9 | 0.64 | 0.20 | - | - | - | | ± | | | | | | | | | - | ++ | - | |
| exo-glucanase | $5.5 \times 10^{-4}$ | 0.11 | 0.19 | - | - | - | - | ± | ± | - | - | - | - | - | 1) | 2) | - | - | - | |
| endo-glucanase | 7.7 | 1.85 | 0.10 | + | ± | - | - | ± | - | - | - | ± | ++ | 3) | 4) | 5) | - | - | - | |
| galactanase | 3.0 | - | 0.90 | - | - | - | - | ++ | | | | | | | | | - | - | | + |
| exo-arabinanase | 0.7 | 3.98 | 1.90 | ± | + | ++ | | - | | | | | | | | | - | - | | |

*- no activity
± weak activity
+ intermediate activity
++ strong activity

1) $2.7 \times 10^{-3}$ U/ml
2) $5.5 \times 10^{-4}$ U/ml
3) 0.48 U/ml
4) 7.7 U/ml
5) $6.9 \times 10^{-2}$ U/ml.

## 5.3. *Results*

### 5.3.1. *Quantity and composition of WIS from fresh tomatoes*

The quantity and composition of WIS prepared from fresh ripe tomatoes is presented in Table 30. A large difference in WIS quantity is found between the two varieties. WIS is characterized by the presence of large amounts of cellulose, anhydrogalacturonic acid and protein. The pectin present in the WIS of fresh tomatoes shows an intermediate degree of esterification.

Table 30: Quantity and composition of WIS prepared from fresh tomatoes (%w/w).

| Variety | H30 | | H1361 | |
|---|---|---|---|---|
| % WIS | 0.74 | | 1.06 | |
| Rhamnose | 1.6 | | 1.5 | |
| Arabinose | 1.5 | | 3.3 | |
| Xylose | 3.8 | | 4.6 | |
| Mannose | 2.4 | | 4.0 | |
| Galactose | 3.6 | | 3.6 | |
| Glucose | 29.5 | | 32.3 | |
| Total n.s.* | | 42.4 | | 49.3 |
| AGA | | 20.6 | | 19.0 |
| DE | 58 | | 59 | |
| Protein | | 16.7 | | 14.5 |
| Cellulose | 34.1 | | 33.4 | |
| Moisture | | 6.6 | | 11.9 |
| Total | | 86.3% | | 94.7% |

* n.s. = neutral sugars

### 5.3.2. *Microscopic appearence of WIS in tomato juice*

In tomato juice the fraction of WIS comprises only 15-20% of all tomato solids. WIS consists mainly of whole tomato cells varying in diameter between 50 µm and 500 µm. These cells occur alone or sometimes in clusters. Furthermore fragments of vascular bundles from both placental tissue and radial and inner walls of the pericarp are present. Finally small pieces of skin material can be found. (Fig. 28). These results confirm data presented by Reeve et al. (1959).

### 5.3.3. *General composition of WIS, pectin, hemicellulose and cellulose prepared from tomato juice*

Table 31 shows the chemical composition of both WIS and WIS fractions for hot break juice of the varieties 318 and 208. As far as the WIS is concerned the two varieties mainly differ in amount of WIS and in the % of arabinose and galactose within this WIS. With the exception of the AGA and protein contents the composition of tomato juice WIS is quite similar to the composition of WIS from fresh tomatoes.
The pectin fraction, in general 40-45% of the WIS, contains large amounts of AGA and protein. Approximately 25-35% of the arabinose and galactose present in the WIS is found as a constituent of the pectin fraction.
The hemicellulose consists mainly of glucose, xylose, mannose and protein. Approximately 50% of the arabinose and galactose and 15-20% of the glucose present in the WIS forms part of the hemicellulose fraction. Depending on the variety the hemicellulose fraction comprises 25-30% of tomato juice WIS. Most of the glucose present in WIS is found in the cellulose fraction. Surprisingly this fraction also contains small amounts of AGA and even ± 15% of the arabinose present in the WIS. Moreover trace amounts of other neutral sugars are found. In general tomato juice WIS contains 30-35% cellulose.

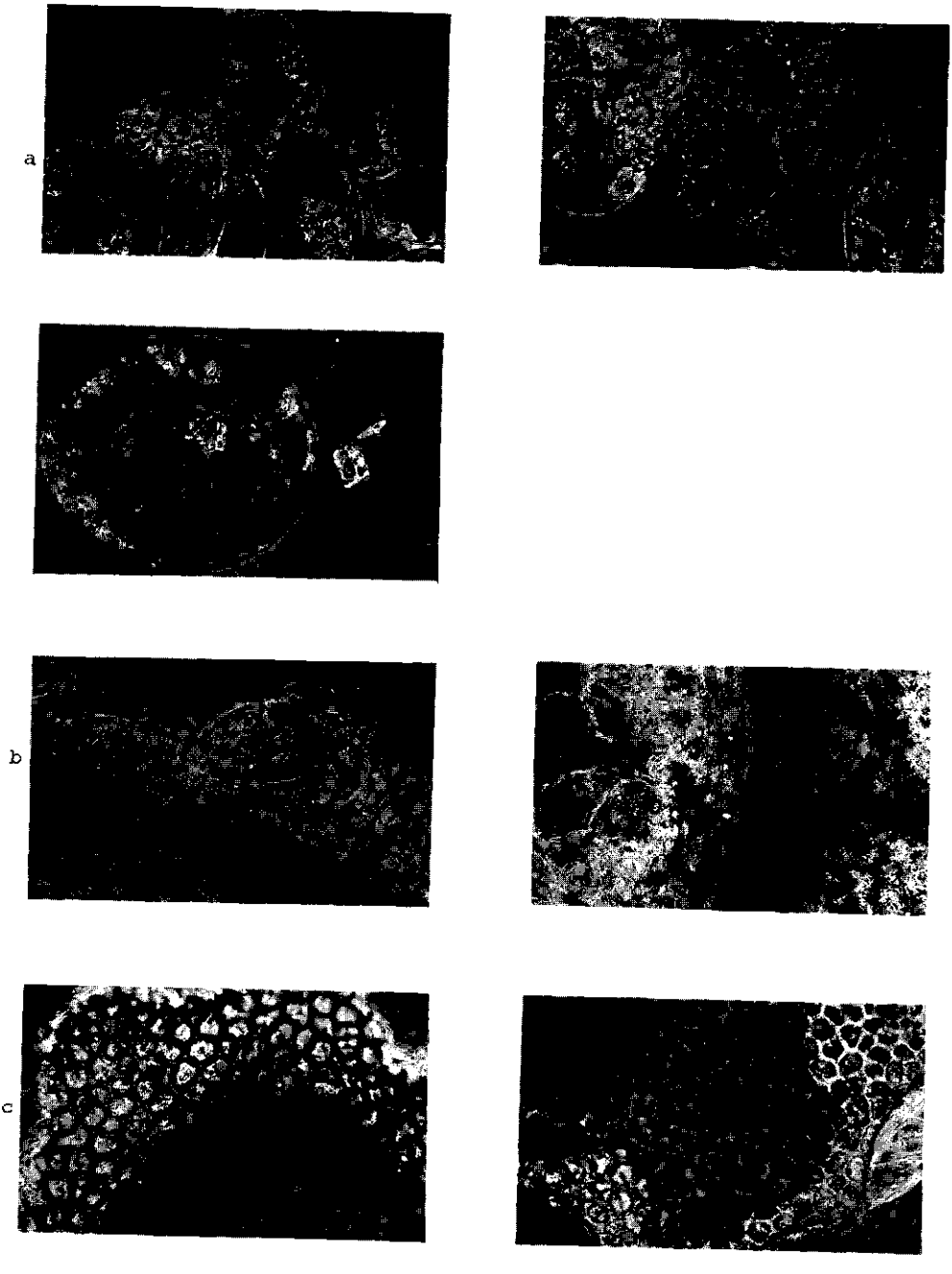



Fig. 28: The WIS of tomato juice. a: whole tomato cells, b: fragments of vascular bundles, c: fragments of skin material (magnification 61.4x).

Table 31: Chemical composition of both WIS and WIS fractions for hot break juice of varieties 318 and 208.

| | 318 | | | | | | | 208 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WIS | PECTIN | | HEMICELLULOSE | | CELLULOSE | | WIS | PECTIN | | HEMICELLULOSE | | CELLULOSE | |
| | weight % | weight % | % of total | weight % | % of total | weight % | % of total | weight % | weight % | % of total | weight % | % of total | weight % | % of total |
| Rham. | 1.0 | 1.7 | 72 | 1.4 | 44 | 0.1 | 9 | 1.0 | 1.3 | 52 | 0.9 | 33 | 0.2 | 5 |
| Ara. | 1.2 | 1.0 | 33 | 2.1 | 56 | 0.5 | 13 | 1.9 | 1.8 | 34 | 3.0 | 50 | 1.0 | 17 |
| Xyl. | 4.3 | 1.3 | 12 | 11.2 | 74 | 0.5 | 3 | 4.1 | 1.0 | 8 | 10.3 | 81 | 0.8 | 6 |
| Mann. | 3.3 | 0.4 | 0 | 8.3 | 77 | 0.8 | 7 | 3.0 | 0 | 0 | 8.1 | 92 | 0.5 | 5 |
| Gal. | 2.8 | 1.7 | 25 | 4.6 | 51 | 0.5 | 5 | 3.9 | 3.0 | 26 | 6.5 | 52 | 0.9 | 7 |
| Gluc. | 31.5 | 0.8 | 1 | 16.7 | 15 | 80.5 | 76 | 30.8 | 0.6 | 1 | 19.1 | 20 | 75.6 | 80 |
| AGA | 13.2 | 28.2 | 85 | - | - | 1.6 | 3 | 15.0 | 32.4 | 78 | - | - | 2.5 | 5 |
| Protein | 20.9 | 28.3 | 54 | 22.3 | 33 | 0 | 0 | 20.8 | 26.3 | 46 | 17.4 | 27 | 1.2 | 2 |
| Cellulose | 32.1 | 1.0 | 1 | 0.1 | 0 | 76.7 | 69 | 31.7 | 1.5 | 1 | 2.3 | 2 | 74.2 | 73 |
| Moisture | 9.2 | - | - | - | - | - | - | 7.0 | - | - | - | - | - | - |
| Total | 87.4 | 63.4 | 39.7* | 66.6 | 31.1* | 84.5 | 29.0* | 87.5 | 66.4 | 36.1* | 65.3 | 31.9* | 82.7 | 31.2* |

WIS % : 318 = 1.01%
208 = 1.14%
DE WIS AGA: 318 = 42.4
208 = 46.6

* expressed as % of total WIS

N.B.: Data for neutral sugars are a mean of triplicate determinations, other data are a mean of duplicate determinations.

## 5.3.4. *Composition and structural features of WIS pectin*

### *Fractionation of WIS pectin*

The pectin present in WIS can be fractionated according to solubility by succesive extractions with 0.2% ammonium oxalate, 0.05 N HCl, both at 60°C, and finally with 0.05 N NaOH + 5 mM EDTA at 4°C. The distribution of the pectin between oxalate, HCl and NaOH soluble fractions is influenced by the successive processing steps used in the production of tomato paste from fresh tomatoes: as indicated in Table 32. As a result of heating during a regular hot break process and subsequent heat processing, much of the HCl soluble pectin is solubilized causing a decrease of both the % of total pectin as well as the percentage of galacturonic acid in the WIS. The solubilized pectin is highly esterified as is indicated by the drop in degree of esterification of the unsolubilized pectin. Concentration causes a further increase in solubilization of WIS-AGA. Comparable results were obtained for tomato, juice and paste of variety H1361.

Table 32: Effect of tomato processing on the fractionation pattern of pectin (variety H30).

| | total pectin fraction (% of WIS) | % AGA in WIS | % of total pectin fraction | | |
|---|---|---|---|---|---|
| | | | oxalate | HCl | NaOH |
| tomato | 43.1 | 14.6 (58) | 20.7 | 25.6 | 53.7 |
| juice | 41.3 | 10.9 (29) | 28.4 | 3.3 | 68.2 |
| paste | 40.8 | 9.3 (28) | 34.3 | 7.0 | 58.7 |

in parentheses the DE of the pectin

Table 33 shows the effect of tomato processing on the sugar composition of the HCl soluble pectin fractions of two varieties. The highly esterified pectin, solubilized as a result of processing, obviously contains only minor amounts of the neutral sugars arabinose and galactose. This table also stresses the large differences which may exist in composition of

pectin or pectin fractions from different varieties.

Table 33: Sugar composition (Mol%) of HCl soluble pectin fraction as influenced by processing (varieties H30, H1361).

| | tomato | | juice | | paste | |
|---|---|---|---|---|---|---|
| | H30 | H1361 | H30 | H1361 | H30 | H1361 |
| Rham. | 7.6 | 5.4 | 7.2 | 8.1 | 8.4 | 7.7 |
| Ara. | 4.1 | 15.9 | 11.1 | 21.8 | 9.8 | 22.4 |
| Xyl. | 1.1 | 1.0 | 1.5 | 1.8 | 2.1 | 2.5 |
| Man. | - | 0.1 | - | 0.4 | 0.2 | 0.9 |
| Gal. | 4.4 | 3.6 | 9.5 | 9.4 | 8.0 | 10.0 |
| Gluc. | 0.9 | 1.1 | 3.5 | 4.2 | 4.9 | 8.7 |
| AGA | 81.9 | 72.9 | 67.2 | 54.3 | 66.6 | 47.8 |

The results for the fractionation of the WIS pectin fraction of non-pasteurized hot break juice of variety H30 are presented in Table 34. The ammonium oxalate soluble pectin fraction is characterized by a relatively low degree of esterification. This fraction contains 23.6% of the AGA, 5.9% of the arabinose and 4.2% of the galactose present in the WIS. The pectin soluble in HCl has a higher DE and is characterized by high amounts of rhamnose, arabinose and galactose. 32.7% of WIS-arabinose, 19.9% of WIS-galactose and 41.0% of WIS-AGA forms part of the HCl soluble pectin. Oxalate soluble pectin and HCl soluble pectin together comprise approximately 65% of the AGA present in the WIS.
Another 30.6% of WIS-AGA is bound in such a way that treatment with diluted alkali is necessary to extract it from the tomato cell wall. On a percentage basis the alkali soluble fraction contains relatively small amounts of AGA, in contrast to the high amounts of protein (60% of total WIS protein) which are found. Together the pectin fractions contain 95% of the AGA, 70% of the rhamnose, 49% of the arabinose, 39% of the galactose but also 10% of the xylose present in WIS.

Table 34: Composition of WIS and pectin fractions for non-pasteurized H30 hot break juice.

| | WIS | 0.2% ammonium oxal. | 0.05 N HCl | 0.05 N NaOH + 5 mM EDTA |
|---|---|---|---|---|
| Rha. | 1.2 | 2.5 (5.7) | 3.7 (6.6) | 0.8 |
| Ara. | 1.6 | 1.1 (2.8) | 4.5 (8.8) | 0.7 |
| Xyl. | 4.2 | 1.2 (3.0) | 1.0 (2.0) | 0.8 |
| Man. | 3.3 | 0.5 (1.0) | - - | - |
| Gal. | 3.7 | 1.8 (3.7) | 6.4 (10.2) | 2.1 |
| Gluc. | 31.7 | 1.9 (3.9) | 1.5 (2.4) | 0.7 |
| AGA | 13.3 | 42.2 (79.9) | 47.6 (70.0) | 15.1 |
| DE | 47 | 34 | 55 | - |
| protein | 19.5 | 26.2 | 6.5 | 43.9 |
| moisture | 6.7 | 12.8 | 10.0 | 9.3 |
| % of juice | 1.08 | 0.08 | 0.124 | 0.290 |
| % of total pectin | - | 16.2 | 25.1 | 58.7 |
| % of total AGA | - | 23.6 | 41.0 | 30.6 |

* in parentheses Mol% sugar composition.

*Gelpermeation chromatography of pectin fractions*

Figures 29a and 29b show the elution profiles on Sephacryl S-200 and Sephacryl S-500 for the ammonium oxalate soluble pectin fraction. The pectin in this fraction has a heterogeneous molecular weight distribution. The apparent molecular weight of the pectin molecule eluting in the included volume (S-500 column, Fig. 29b) is estimated at 40,000-80,000; the amount of AGA in this molecule equals 7% of total WIS-AGA.
Elution of the HCl soluble pectin fraction on a Sephacryl S-500 column results in its separation into two types of pectin molecules, of which one has a molecular weight much higher than the oxalate soluble pectins (Fig. 29c). The higher molecular weight fraction (pool I) contains 15-16% of the WIS-AGA, the other fraction (pool II) 13-18% of WIS-AGA. Pool II was fractionated on a S-500 column while eluted with water (Fig. 29d). Due to

charge effects a pectin with small amounts of neutral sugars (pool III) was separated from the remaining pectin.

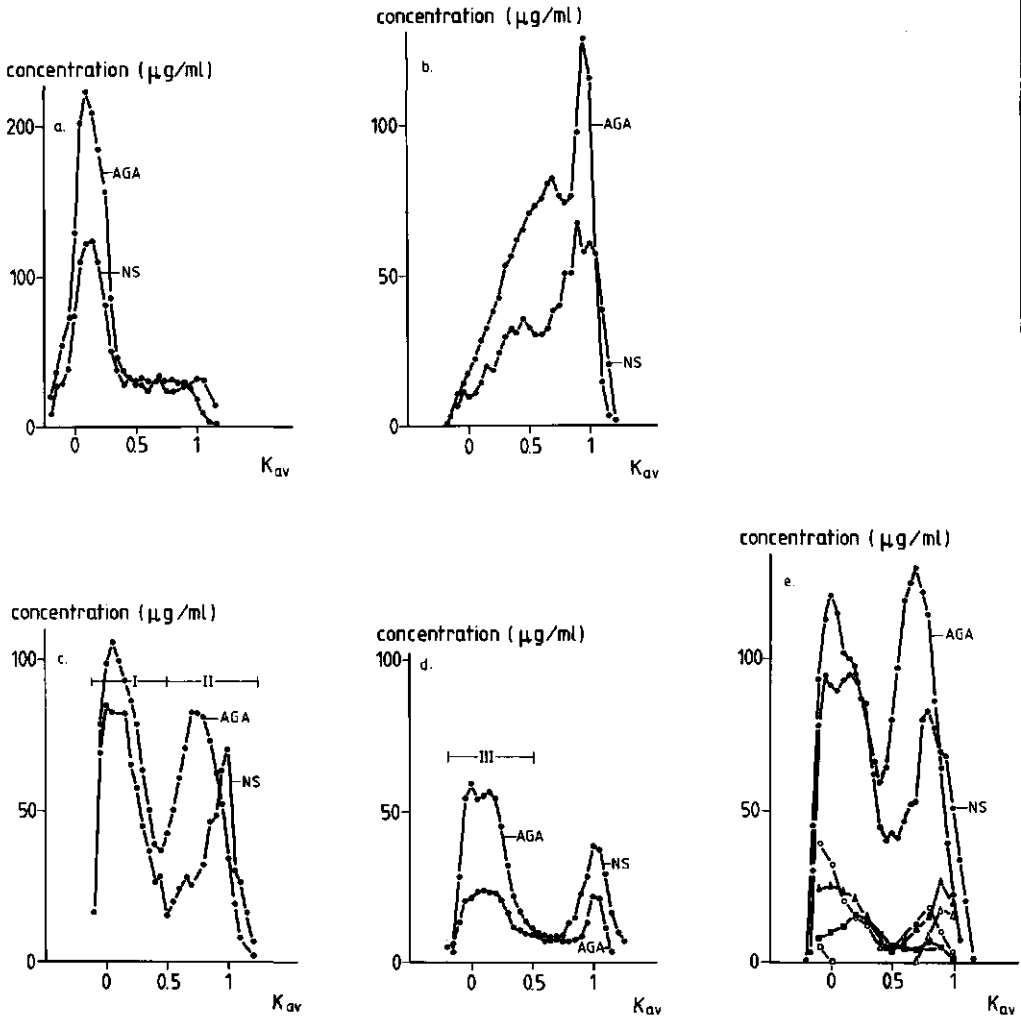



Fig. 29: Elution patterns from gel permeation chromatography of pectin fragments of tomato juice.
a. 10.7 mg oxalate soluble pectin; S-200 eluted with buffer
b. 9.2 mg oxalate soluble pectin; S-500 eluted with buffer
c. 9.7 mg HCl soluble pectin; S-500 eluted with buffer
d. Pool II Fig. 29c (1.31 mg AGA); S-500 eluted with water
e. 10.2 mg HCl soluble pectin; S-500 eluted with buffer
■ Rhamnose, ▲ Arabinose, ◘ Xylose, ○ Galactose, △ Glucose.

Table 35 shows the sugar composition of this fraction. The amount of AGA present in this molecule corresponds to 10% of the WIS-AGA.

Table 35: Sugar composition in Mol% of pool III (Fig. 29d)

| | |
|---|---|
| Rham. | 3.6 |
| Ara. | 3.0 |
| Xyl. | 1.1 |
| Man. | 0.3 |
| Gal. | 5.1 |
| Gluc. | 1.5 |
| AGA | 85.2 |

The elution of the HCl soluble pectin on Sephacryl S-500 was repeated; from each fraction the AGA content and neutral sugar composition was determined. (Fig. 29e). Most of the arabinose and galactose co-eluted with the higher molecular weight pectin fragment. This fragment also contained minor amounts of xylose. Part of the arabinose and glucose eluted in the tail of the included peak; obviously these sugars are not connected to the AGA of the lower molecular weight pectin fragment.

### 5.3.5. *Composition and structural features of tomato hemicellulose*

The sugar composition of the hemicellulose fraction was found to be less sensitive to tomato processing than the sugar composition of the pectin fraction. The composition of hemicellulose fractions is given in Table 36.

Hemicellulose isolated from AIS prepared from fresh tomatoes was fractionated on DEAE-cellulose (Fig. 30). This resulted in various fractions with different sugar composition (Table 37). Sugar compositions derived from methylation analysis corresponded well with these figures. Fractions C, E and F are high in xylose content; fractions A, B and D contain a high proportion of glucose; fractions A and B moreover contain relatively high amounts of mannose.

From methylation analysis (Table 38) it appears that the xylose residues in fractions C, E and F are predominantly 1,4-linked and very likely originate from β-1,4-xylan. In this connection fraction E, containing 42% of the xylose present in hemicellulose, is the most important fraction.

Table 36: Effect of tomato processing on the sugar composition (Mol%) of the hemicellulose fraction. (varieties H30 and H1361).

| | H30 | | | H1361 | | |
|---|---|---|---|---|---|---|
| | tomato | juice | paste | tomato | juice | paste |
| Rham. | 0.9 | 1.0 | 1.6 | 0.8 | 0.8 | 1.0 |
| Ara. | 5.2 | 5.6 | 6.0 | 6.2 | 5.5 | 5.6 |
| Xyl. | 24.0 | 24.5 | 26.8 | 30.0 | 30.2 | 29.3 |
| Man. | 16.5 | 15.8 | 17.9 | 17.8 | 17.9 | 17.2 |
| Gal. | 9.4 | 9.7 | 9.6 | 9.7 | 9.7 | 9.7 |
| Gluc. | 38.4 | 37.9 | 33.3 | 29.5 | 31.6 | 31.9 |
| AGA | 5.5 | 5.4 | 4.7 | 5.8 | 4.4 | 5.3 |

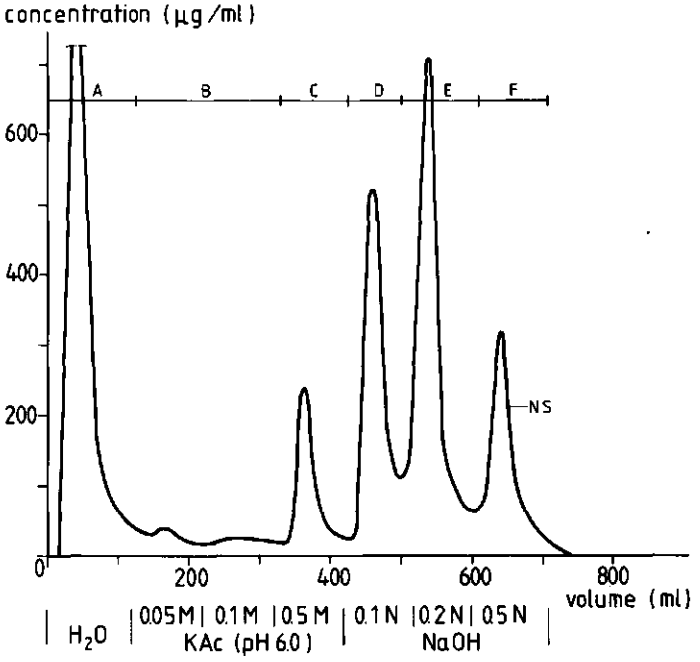



Fig. 30: Fractionation of tomato hemicellulose on DEAE-cellulose.

Table 37: Sugar composition of tomato hemicellulose (variety H2826) and subfractions obtained by DEAE-cellulose chromatography.

| Fractions | % of total hemicell. sugars | composite sugars (Mol%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rha./Fuc. | Ara. | Xyl. | Man. | Gal. | Gluc. | AGA |
| Hemicellulose extract | 100% | - | 3.4 | 28.3 | 19.0 | 9.5 | 36.6 | 3.2 |
| DEAE-cellulose fractions: | | | | | | | | |
| A) $H_2O$ | 39.7 | - | 3.0 (34.1) | 12.7 (17.3) | 24.6 (49.9) | 12.1 (49.1) | 46.9 (49.4) | 0.7 (8.4) |
| B) 0.1 M KAc pH 6 | 1.5 | - | 4.0 (1.7) | 16.7 (0.9) | 24.4 (1.9) | 12.6 (2.0) | 40.8 (1.6) | 1.4 (0.6) |
| C) 0.5 M KAc pH 6 | 3.3 | - | 3.5 (3.5) | 55.7 (6.8) | 4.1 (0.7) | 6.1 (2.2) | 13.1 (1.2) | 17.4 (18.7) |
| D) 0.1 N NaOH | 7.7 | - | 5.0 (11.5) | 34.7 (9.6) | 3.3 (1.4) | 6.0 (5.0) | 49.6 (10.6) | 1.4 (3.4) |
| E) 0.2 N NaOH | 15.2 | - | 1.3 (6.3) | 72.4 (42.2) | 3.7 (3.2) | 1.9 (3.3) | 13.4 (6.0) | 7.3 (37.7) |
| F) 0.5 NaOH | 5.0 | - | - | 59.1 (11.0) | 16.5 (4.6) | 3.7 (2.1) | 14.3 (2.1) | 6.4 (10.5) |
| Residue | 5.8 | - | 4.2 (7.2) | 29.0 (6.0) | 8.6 (2.6) | 5.9 (3.6) | 49.5 (7.9) | 2.7 (4.9) |
| Recovery of fractions | 78.2 | - | 64.3 | 93.8 | 64.3 | 67.3 | 78.8 | 84.2 |

Figures in parentheses: sugars expressed as % of amounts present in original hemicellulose extract.

Table 38: Glycosyl linkage composition of tomato hemicellulose fractions.

| Monosaccharide | Deduced glycosyl linkage | Occurence of type of glycosyl linkage per sugar unit in % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | Residue |
| Araf. | terminal | 100 | 100 | 100 | 83 | 100 | | 100 |
| | 1,2,3,5- | | | | 17 | | | |
| Xylp. | terminal | 51 | 12 | 6 | 42 | 7 | 4 | 45 |
| | 1,4- | 5 | ↕ 35 | 82 | 25 | 81 | 75 | 13 |
| | 1,2- | 28 | | | 24 | | | 33 |
| | 1,2,4- | | | 4 | 4 | 8 | 10 | |
| | 1,2,3- | 16 | | 0.3 | 4 | 1 | 1 | |
| | 1,3,4- | | | 2 | | 3 | 4 | |
| | 1,2,3,4- | | 53 | 7 | 1 | | 6 | 9 |
| Manp. | 1,4- | 69 | 74 | 100 | 82 | 80 | 100 | 73 |
| | 1,4,6- | 27 | 26 | | 18 | 20 | | 27 |
| | 1,2,4,6- | 4 | | | | | | |
| Galp. | terminal | 70 | 100 | | 100 | | 100 | 21 |
| | 1,4- | 26 | | 100 | | | | 52 |
| | 1,3,4- | | | | | | | 27 |
| | 1,3,4,6- | 4 | | | | 100 | | |
| Glucp. | terminal | 5 | | 23 | | 8 | 12 | |
| | 1,4- | 61 | 39 | 39 | 49 | 57 | 74 | 47 |
| | 1,4,6- | 26 | 19 | 25 | 43 | 35 | 14 | 33 |
| | 1,2,4- | 4 | | | | | | |
| | 1,3,4- | | | | | | | 3 |
| | 1,2,3,4- | | | | | | | 4 |
| | 1,3,4,6- | 1 | | | 2 | | | 5 |
| | 1,2,3,4,6- | 3 | 42 | 13 | 6 | | | 8 |

p indicates pyranose ringform
f indicates furanose ringform

These three fractions also contain most of the AGA present in the hemicellulose. Fractions A, D and the insoluble residue have high proportions of terminal xylose residues, 1,2-linked xylose residues, and 1,4 as well as 1,4,6-linked glucose residues, as is typical for xyloglucans. Further support for the presence of xyloglucans can be derived from the results presented in Fig. 32b. The presence in fraction A of 1,4-linked glucose, 1,4-linked mannose with branching at C-6, and terminal galactose may point to the occurrence of galactoglucomannans. However, due to the complex composition of these fractions the interpretations, are tentative.

In general the tomato hemicellulose contains 40-50% of the arabinose and galactose present in the WIS. It is noteworthy that all the arabinose residues are terminally linked, indicating the absence of arabinans in the hemicellulose fraction. Approximately 15% of the hemicellulose galactose is 1,4-linked (fraction A, insoluble residue) and may be present as galactans. Gel permeation chromatography on Sephacryl S-500 (Fig. 31), with water as eluent, revealed a high molecular weight uronide containing fraction rich in xylose (Fig. 31a, b), and an included fraction with lower molecular weight polymers particularly rich in mannose and glucose (Fig. 31c).

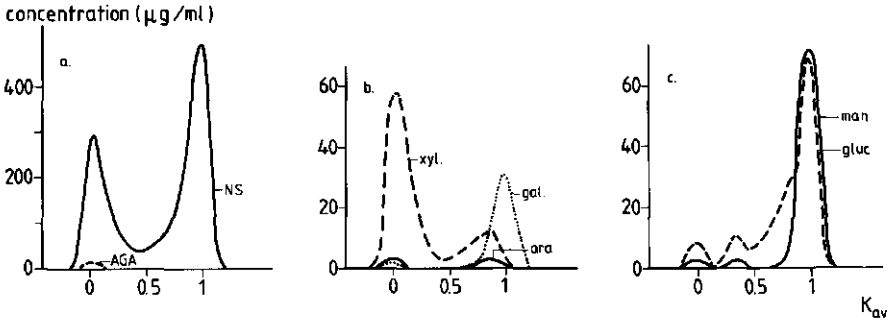



Fig. 31: Gel permeation chromatography of tomato hemicellulose on Sephacryl S-500, eluent distilled water
a. Elution pattern for AGA and total neutral sugars (NS)
b. Elution pattern for arabinose (ara), xylose (xyl) and galactose (gal)
c. Elution pattern for mannose (man) and glucose (gluc)

After treatment of the hemicellulose fraction with purified endo-β -1,4-glucanase, the void peak almost completely shifts to the included fraction (Fig. 32a).The remaining polysaccharides in the void peak contain only xylose and uronic acid. It is not known whether this uronic acid is galacturonic acid or methyl-O-glucuronic acid. The fractionation pattern of the S-500 included pool on Bio-Gel P-2 is shown in Fig. 32b. The composition of the intermediate oligomeric fragments is typical for xyloglucan fragments. Presumably both xylans and xyloglucans are degraded by the endo-glucanase used. The multisubstrate specificity of this enzyme was shown by Beldman et al. (1986). The high mannose content in the P-2

void, in particular in the tail of the peak is also striking. The dimer fraction is high in xylose and arabinose. Xylose and glucose are the main monomeric sugars found.

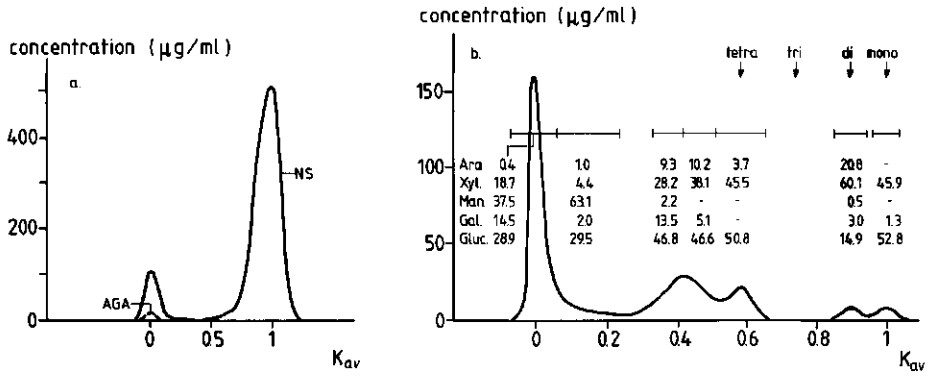



Fig. 32: Elution patterns for endo-glucanase treated tomato hemicellulose.
a. S-500 eluted with buffer
b. included pool Fig. 32a; P-2 eluted with water

### 5.3.6. *Composition and structural features of the fragments solubilized from WIS by enzymes*

A second approach to the study of the composition and structure of tomato WIS is the analysis of WIS fragments solubilized by purified pectolytic, cellulolytic and hemicellulolytic enzymes.
The characteristics for the purified enzymes are given in section 5.2.4. (Table 29); the chemical composition of the WIS is shown in Table 34. Table 39 presents the results of these studies. It is obvious that none of the enzymes has been able to solubilize substantial amounts of protein from the WIS.
As a control experiment WIS was suspended in 0.05 M sodium acetate buffer pH 5.0, the same buffer as used for the enzyme experiments. This treatment resulted in the solubilization of some sugars. Gel permeation chromatography of these sugars on Sephacryl S-200 indicated the presence of high molecular weight pectin fragments as well as low molecular weight neutral sugar containing fragments (Fig. 33). The only neutral sugars found in the low molecular weight fragments were glucose and mannose.

Table 39: Amount of sugars, AGA and protein solubilized from WIS as a result of enzyme action (% of total amount present).

| enzyme | Rham. | Ara. | Xyl. | Man. | Gal. | Gluc. | AGA | Protein |
|---|---|---|---|---|---|---|---|---|
| Control | 4.0 | 5.4 | 1.2 | 1.0 | 5.0 | 1.6 | 7.5 | 0.0 |
| PL | 36.4 | 45.6 | 9.2 | 2.0 | 23.4 | 2.7 | 58.4 | 4.1 |
| PG | 21.8 | 15.0 | 7.2 | 0.2 | 9.6 | 0.2 | 54.9 | 6.7 |
| PE + PG | 30.1 | 49.1 | 10.8 | 0.0 | 28.0 | 0.4 | 83.9 | 5.0 |
| exo-glucanase + endo-glucanase | 21.4 | 36.2 | 48.0 | 45.1 | 21.8 | 42.6 | 22.8 | 4.5 |
| exo-g + endo-g + PE + PG | 42.7 | 68.7 | 69.0 | 46.5 | 47.2 | 49.4 | 77.3 | 5.5 |
| exo-g + endo-g + PL | 33.0 | 91.0 | 58.5 | 40.8 | 44.5 | 40.2 | 70.8 | 0.0 |
| galactanase | 18.5 | 34.7 | 1.4 | 0.2 | 24.9 | 0.8 | 43.1 | 0.0 |
| exo-arabinanase | 0.0 | 38.1 | 1.3 | 0.9 | 0.0 | 1.2 | 0.0 | 2.5 |
| exo-arabinanase + PL | 28.4 | 52.4 | 5.3 | 0.8 | 16.9 | 2.0 | 40.4 | 0.0 |
| exo-arabinanase + PG | 15.4 | 47.7 | 5.5 | 3.6 | 7.3 | 1.5 | 35.9 | 3.9 |

exo-g = exo-glucanase, endo-g = endo-glucanase.

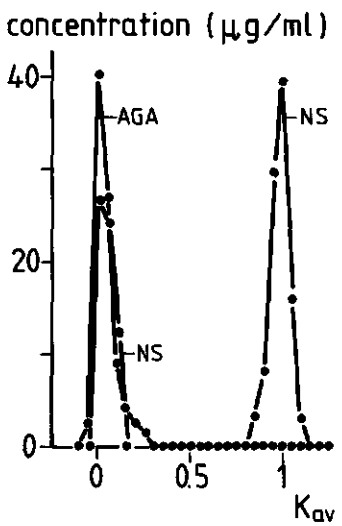



Fig. 33: Elution pattern (S-200, buffer elution) for sugars solubilized from 28.6 mg WIS as a result of incubation with buffer.

*Fragments solubilized by PL*

Apart from having a slight activity on potato galactan the pectinlyase used in this study can be considered a pure enzyme. Large amounts of AGA, arabinose, rhamnose and galactose were released from WIS as a result of incubation with pectinlyase. The ratio AGA/neutral sugars in the reaction liquid was 2.0; the degree of polymerisation (total sugar/reducing sugar ratio) of the solubilized fragments was estimated at 6.
The fractionation pattern of the reaction liquid on Sephacryl S-200 is shown in Fig. 34a. Table 40 gives the sugar composition of the fragment voiding the S-200 column in a sharp, narrow peak. (pool I).

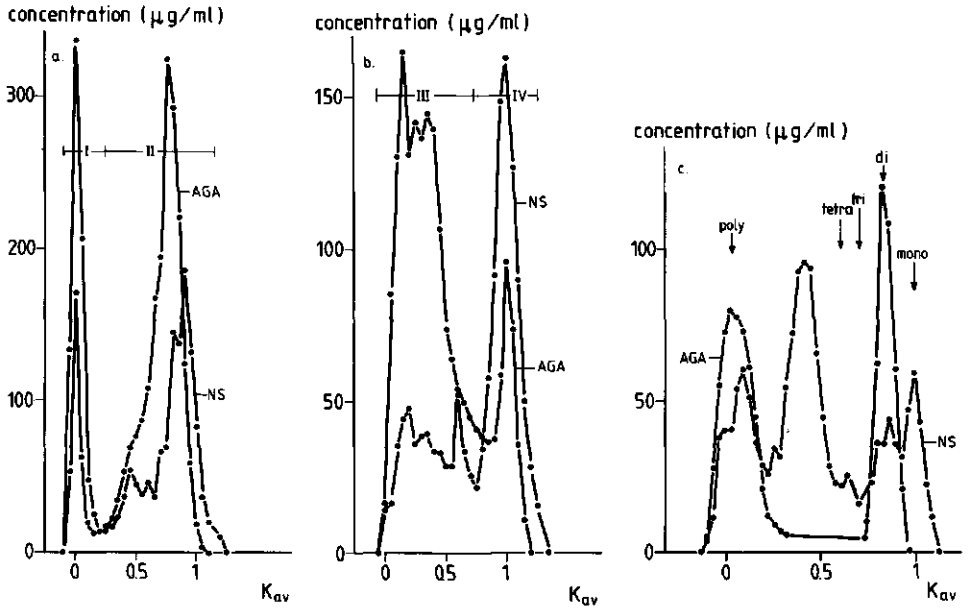



Fig. 34: Elution patterns for fragments solubilized from 58.9 mg tomato WIS as a result of incubation with PL.
a. Total reaction liquid; S-200 eluted with buffer
b. Pool II Fig. 34a (3.7 mg AGA); S-200 eluted with water
c. Pool IV Fig. 34b (0.9 mg AGA); P-2 eluted with water

Since the enzyme was pure it can be assumed that the neutral sugars are bound to the pectin fragment. It can be concluded that PL solubilizes a high molecular weight ($\geq$ 80,000), highly branched pectin fragment containing 9% of the AGA present in WIS. Noteworthy is the presence of xylose in this fragment.

Table 40: Sugar composition (Mol %) of pool I (Fig. 34a).

| | Mol% | % of total amount present in WIS |
|---|---|---|
| Rham. | 10.5 | 21 |
| Ara. | 18.7 | 26 |
| Xyl. | 9.9 | 5 |
| Gal. | 21.3 | 16 |
| AGA | 38.3 | 9 |

The fractionation pattern of the reaction liquid on Sephacryl S-200 (Fig. 34a) also indicates that the bulk of the solubilized AGA is not bound to the low molecular weight neutral sugar containing fragments. Pool II was concentrated and fractionated on Sephacryl S-200 while eluted with water (Fig. 34b). Due to charge effects uronide containing fragments were separated from neutral sugar containing fragments. The elution profiles shown in Figures 34a and 34b indicate that approximately 40% of the AGA present in WIS is solubilized by PL in the form of intermediate sized (MW ± 10,000-40,000) polymers, containing small amounts of neutral sugars (Table 41).

Table 41: Sugar composition (Mol%) of pool III after fractionation on Sephacryl S-200 (Fig. 34b).

| | Mol % | % of total amount present in WIS |
|---|---|---|
| Rham. | 1.7 | 8 |
| Ara. | 1.8 | 6 |
| Xyl. | 1.5 | 2 |
| Man. | 0.2 | |
| Gal. | 2.0 | 3 |
| Gluc. | 3.5 | |
| AGA | 89.2 | 40 |

The neutral sugar containing fragments (pool IV, Fig. 34b) were fractionated on Bio-Gel P-2 (fig. 34c). Only 5% of the AGA present in WIS is degraded to monomer/dimer as a result of PL action; 8% of the AGA is degraded to oligomeric sized fragments. The monomeric fraction contains glucose and arabinose (3% of total present in WIS); the dimeric fraction only glucose. Most of the neutral sugars were found in a fraction with a degree of polymerisation between 5 and 10. This fraction consists largely of glucose (56.9 Mol%, 1.2% of total WIS-glucose) and xylose (23.3 Mol%, 3% of total WIS-xylose).

*Fragments solubilized by PG*

As has been shown in Table 29 the polygalacturonase used in this study was pure except for having a slight activity on β-1,4-xylan. The amount of AGA solubilized as a result of PG action is comparable to the amount solubilized by PL treatment (Table 39).

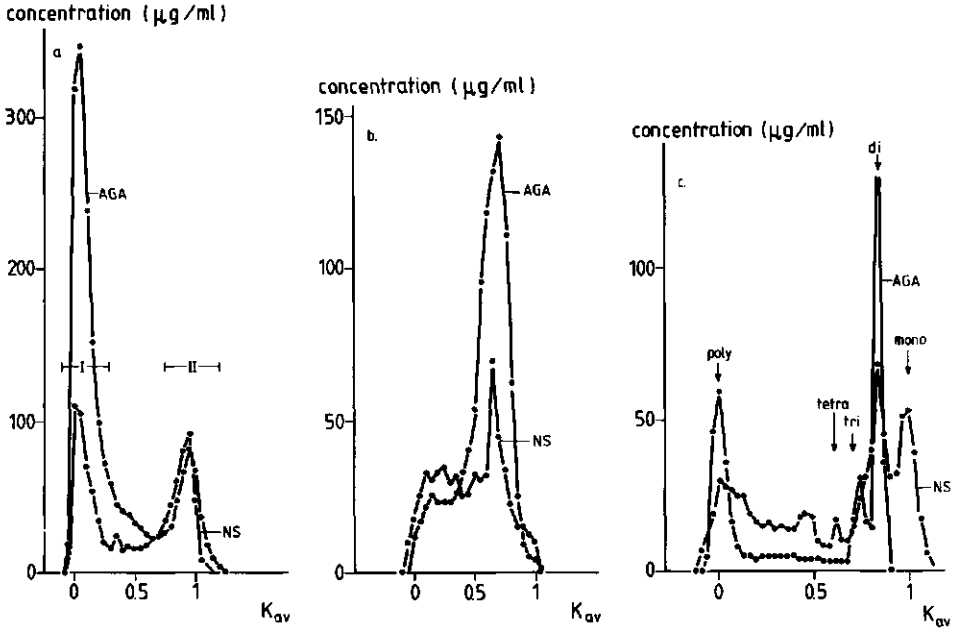



Fig. 35: Elution patterns for fragments solubilized from 56.4 mg tomato WIS as a result of incubation with PG.
a. Total reaction liquids; S-200 eluted with buffer
b. Pool I, Fig. 35a (2.3 mg AGA); S-500 eluted with buffer
c. Pool II, Fig. 35a (0.7 mg AGA); P-2 eluted with water

However, the amounts of arabinose and galactose solubilized, respectively 15.0% and 9.6% of the quantities present in WIS, are considerably lower than the quantities released by PL action.

The ratio AGA/neutral sugars in the reaction liquid was 5; the average degree of polymerization of the solubilized fragments was estimated at 5.

Figure 35a shows the fractionation pattern of the reaction liquid on Sephacryl S-200. 35% of the AGA present in WIS (67% of total solubilized by PG) is solubilized as high molecular weight fragments.

Pool I was concentrated and fractionated on Sephacryl S-500 (Fig. 35b). The elution profiles shown in Figures 35a and 35b indicate that 24% of the WIS-AGA is solubilized as fragments with an apparent molecular weight between 40,000 and 80,000. The molar sugar composition of these fragments, given in Table 42, closely resembled the sugar composition of the unfractionated reaction liquid, except for the amount of xylose present.

Table 42: Sugar composition (Mol %) of fragments with a MW of ± 40,000-80,000 released by PG (Fig. 35b).

| | Mol% | % of total amount present in WIS |
|---|---|---|
| Rham. | 3.9 | 10 |
| Ara. | 4.3 | 8 |
| Xyl. | 1.6 | 1 |
| Man. | - | |
| Gal. | 5.3 | 5 |
| Gluc. | 1.8 | |
| AGA | 83.2 | 24 |

The degree of esterification of the pectin in the reaction liquid was found to be 58, which is higher than the DE of the total WIS-pectin. Obviously this high DE is unfavourable for further PG catalysed degradation of solubilized pectin.

Fig. 35a shows that only 10% of the WIS-AGA is degraded to low molecular weight fragments (pool II) as a result of PG action. Fractionation of these fragments on Bio-Gel P-2 (Fig. 35c) revealed that 4% of this AGA is

degraded presumably to monomer and dimer and 3% to oligomers. The only neutral sugars determined in the Bio-Gel P-2 fractions were xylose, glucose and a very small amount of monomeric galactose. The presence of xylose and glucose may be explained by the residual xylanase activity of the enzyme.

*Fragments solubilized by PE + PG*

By the simultaneous action of PE and PG, 84% of the AGA present in WIS could be solubilized; only 9% remained unsolubilized. Compared to the effect of PG action, the combination of PE and PG induced the solubilization of large amounts of arabinose and galactose. The ratio AGA/neutral sugars for PE + PG was 3.3, compared to 2.0 for PL and 5.0 for PG. Apparently the arabinose and galactose containing fragments solubilized by PE + PG, but not by PG alone, are part of highly esterified regions in the WIS pectin. Since these highly esterified regions were also released by PL but not by PG alone they are apparently not connected to the cell wall matrix by means of low esterified pectin regions.

*Fragments solubilized by endo-glucanase + exo-glucanase*

The combination of endo- and exo-glucanase (fractions $III_1$ and exo III, Beldman et al. 1985) released large amounts of glucose, xylose and mannose containing polymers. Also noteworthy is the solubilization of 22.8% of the WIS-AGA. Because of the absence of pectolytic enzyme activity (not found in the crude technical enzyme preparation), these solubilized high molecular weight pectin fragments (as indicated by S-500 gel permeation) were probably bound to solubilized parts of hemicellulose and/or cellulose. Due to small impurities of arabinanases and galactanases and the activity of endo $III_1$ on arabinoxylan it is difficult to draw conclusions about the occurrence of arabinose and galactose molecules in these solubilized pectin fragments. Solubilized neutral sugars (eluting in the included of the S-500 fractionation) were fractionated on Bio-Gel P-2 (Fig. 36). The elution pattern shows four peaks: monomers, dimers and two peaks with a degree of polymerisation between 6 and 10 ($K_{av}$ 0.4 and 0.1 respectively). Glucose sugars were mainly present as cellobiose (27% of WIS-glucose), indicating cellulose degradation and a low cellobiase activity. 6% of the WIS-glucose was present as monomer; the remaining part of the solubilized glucose was found in the fragments with $K_{av}$ 0.1 and 0.4. 17% of the WIS-xylose is

degraded to monomers; 21% to oligomers.

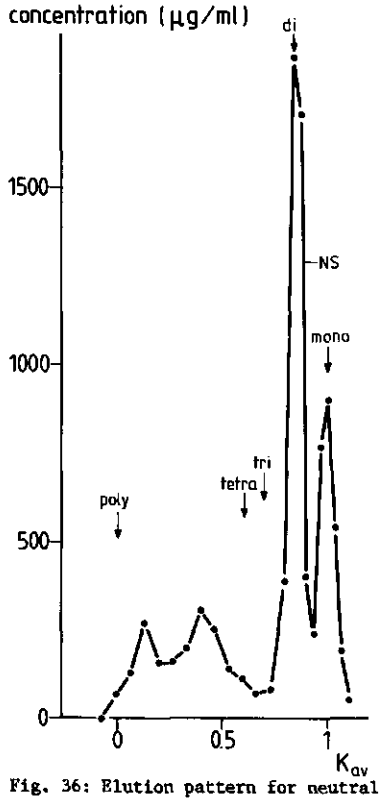



Fig. 36: Elution pattern for neutral sugar containing fragments solubilized from 57.2 mg tomato WIS as a result of incubation with endo- and exo-glucanase. P-2 column, eluted with water.

The peak with $K_{av}$ 0.4 contains 13% of the WIS-xylose, the peak with $K_{av}$ 0.1 8%. Mannose was found as oligomers and dimers; 25% of the WIS-mannose in the peak with $K_{av}$ 0.4, 9% in the peak with $K_{av}$ 0.1 and 6% as dimer.

*Fragments solubilized by PE + PG + endo-glucanase + exo-glucanase*

In general no synergistic effects were observed when endo-glucanase, exo-glucanase, PE and PG were added simultaneously to WIS.
Only for xylose did the combination of enzymes release more sugar than the sum of the separate actions. This could mean that xylans are involved in the binding of pectin to celluloses. The amount of galactose solubilized as

a result of combined pectolytic and cellulolytic enzyme action is the same as the sum of the separate effects. Obviously the pectolytic and cellulolytic enzymes act on different galactose containing polymers. The amounts of rhamnose and arabinose solubilized as a result of the combined addition of cellulases, PE and PG were even less than the combined separate effects. Parts of the rhamnose and arabinose solubilized by glucanases are also solubilized by the combination of PE and PG. The addition of glucanases to PE and PG does not increase the solubilization of AGA; glucanases apparently solubilize parts of WIS pectin also solubilized by PE + PG.

*Fragments solubilized by PL + endo-glucanase + exo-glucanase*

The combined action of glucanases and PL solubilizes 91% of the WIS-arabinose; this is more than the sum of the amounts of arabinose solubilized by glucanases and PL separately. It appears that arabinan chains form a connection between cellulose and pectins. This finding is supported by the presence of 15% WIS-arabinose in the cellulose fraction. The arabinan in this particular arabinan containing pectin segment is not solubilized by PL alone nor by the combined action of glucanases + PE + PG. The amounts of xylose and galactose solubilized by the combined action of glucanases and PL were the sum of the individual amounts solubilized. The amount of AGA solubilized by the combined action of glucanases and PL was less than the sum of the separate effects; part of the amount solubilized by glucanases is also solubilized by PL.

*Fragments solubilized by pure galactanase*

The addition of pure galactanase resulted only in the solubilization of significant amounts of AGA, arabinose, galactose and rhamnose. The purified galactanase was free of PG, PL, xylanase and arabinanase activity and showed no activity on a β-1,3, β-1,6 linked arabinogalactan (Table 29). Fractionation of the reaction liquid on Sephacryl S-200 (Fig. 37a) shows that the AGA is solubilized as high molecular weight fragments. Pool I was concentrated and fractionated on Sephacryl S-500 (Fig. 37b). Figures 37a and 37b show that 21% of the WIS-AGA is solubilized as fragments with an apparent molecular weight between 40,000 and 80,000. The results indicate

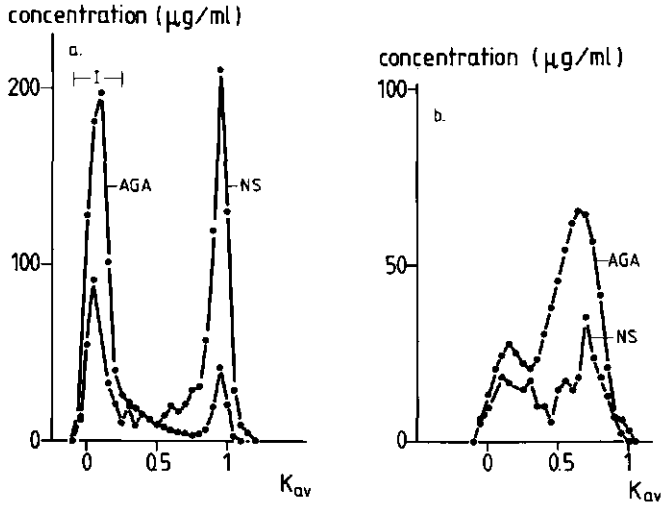



Fig. 37: Elution patterns, for fragments solubilized from 37.1 mg tomato WIS as a result of incubation with pure galactanase.
a. Total reaction liquid; S-200 eluted with buffer
b. Pool I Fig. 37a (1.3 mg AGA); S-500 eluted with buffer

that galactans are important in the binding of pectin to the cell wall matrix. The arabinose solubilized as result of pure galactanase action (34.7% of the WIS-arabinose) is not bound to the high molecular weight AGA. It is found in the included fraction and in the intermediate fraction of the S-200 fractionation (Fig. 37a). Solubilized galactose is mainly present in the included fraction of the S-200 fractionation but is also found in the void. The fact that the arabinan is not found associated with the high MW pectin fragments implies that it was originally part of the galactan polymer.

*Fragments solubilized by exo-arabinanase*

Purified exo-arabinanase is very specific in solubilizing arabinose from WIS (Table 39). The absence of any solubilized galactose, rhamnose and AGA points to the presence of at least 38% of the WIS-arabinose as side chains on other cell wall polymers.

*Fragments solubilized from the residues of enzyme treated WIS*

The WIS residues left after the enzyme incubations were freeze-dried and again incubated with enzymes. The results are shown in Table 43.

The addition of PG to the residue of endo-glucanase + exo-glucanase treated WIS results in the solubilization of 23% of the WIS-AGA. This result confirms the conclusion that endo-glucanase + exo-glucanase solubilize a part of the WIS-AGA which is also solubilized by PG. Gel permeation of the reaction liquid on Sephacryl S-200 shows that approximately 60% of the solubilized AGA is present in the void peak, together with most of the solubilized rhamnose, arabinose and galactose.

PG is not able to solubilize large amounts of AGA from the residue of PL treated WIS; however, when compared to the amount of solubilized AGA, the amounts of solubilized rhamnose, arabinose and galactose are rather high (compare with Table 39: effect of PG alone).

Table 43: Amounts of sugar and AGA solubilized from WIS residues as a result of enzyme action (% of total amount originally present in WIS).

| residue of treatment with: | second enzyme | Rham. | Ara. | Xyl. | Man. | Gal. | Gluc. | AGA |
|---|---|---|---|---|---|---|---|---|
| endo-g + exo-g | PG | 11.3 | 8.2 | 5.5 | 2.9 | 3.7 | 2.8 | 23.0 |
| PL | PG | 13.4 | 10.0 | 6.8 | 1.0 | 6.2 | 0.4 | 15.4 |
| endo-g + exo-g | PL | 36.5 | 36.3 | 5.7 | 2.5 | 17.8 | 3.3 | 46.6 |
| PG | PL | 22.8 | 30.5 | 9.6 | 2.4 | 12.6 | 2.7 | 31.3 |
| galactanase | PL* | 47.5 | 41.6 | 18.8 | 8.5 | 18.8 | 5.3 | 25.9 |

* 0.1 ml enzyme added to 50 mg residue instead of 0.2 ml (Table 29).

Obviously PL has released those parts of the WIS pectin which are low in rhamnose, arabinose and galactose content and which are also liable to PG action.

In contrast to PG, PL is able to solubilize large amounts of AGA from the residue of endo-glucanase + exo-glucanase treated WIS. The molar sugar composition of the reaction liquid and the fractionation pattern on Sephacryl S-200 are very similar to those for the reaction liquid of WIS treated with PL alone. The glucanases and PL obviously attack unrelated

parts of the WIS.

The incubation of the residue of PG treated WIS with PL resulted in the solubilization of 31% of the AGA, 31% of the arabinose, 23% of the rhamnose and 13% of the galactose present in the original WIS. It appears that part of the AGA released by PL can also be released by PG. The pectin segments solubilized by both enzymes are low in rhamnose, arabinose and galactose content.

The addition of PL to the residue of WIS treated with purified galactanase results in the solubilization of 26% of the WIS-AGA, which is accompanied by solubilization of amounts of rhamnose, arabinose and galactose comparable to the amounts solubilized by PL alone. Noteworthy is the solubilization of 19% of the WIS-xylose and 5% of the WIS-glucose; these sugars were also found in the reaction liquid after treatment with PL alone (Fig. 34c).

## 5.4 *Discussion*

The WIS quantities present in tomatoes and tomato juice as determined in our studies ranged from 0.74% to 1.14% on a fresh weight basis; these data are comparable to the values reported in the literature (see section 2.5). Apart from the differences in WIS quantity, the samples mainly differed in % of arabinose and galactose present in the WIS and WIS fractions (Tables 30, 31, 33). It is well known that the amounts of WIS, arabinose and galactose are strongly influenced by variety and level of ripeness (section 2.3.2.).

Based on our data tomato WIS can be fractionated into 40-45% pectin, 25-30% hemicellulose and 30-35% cellulose. Jona & Foa (1979) reported values of respectively 51%, 18% and 31%. Gross (1984), who fractionated the cell walls of the outer pericarp, found 48.3% cellulose in addition to 32.4% pectin and 19.3% hemicellulose. Jona & Foa (1979) had already reported that the cell walls of the outer pericarp were rich in cellulose.

The pectin fraction contained almost all of the AGA, the major part of the protein, but less than 50% of the arabinose and galactose present in the WIS. Surprisingly, the pectin fraction contained 10% of the WIS-xylose. The presence of xylose in cell wall pectin has also been reported by other

authors (de Vries 1981, 1983; Stevens & Selvendran 1984). Mannose and xylose were mainly found in the hemicellulose fraction. This fraction also contained up to 50% of the arabinose and galactose present in WIS, 15-20% of the WIS-glucose and substantial amounts of the protein.
The major part of the glucose present in WIS was found in the cellulose fraction. This fraction also contained 15% of the WIS-arabinose. The presence of arabinose in cellulose fractions is confirmed by Stevens & Selvendran (1980) and Voragen et al. (1983).

The insoluble pectin of non-pasteurized hot break juice could be fractionated into 16.2% oxalate soluble, 25.1% HCl soluble and 58.7% NaOH soluble pectin (Table 34). These fractions contained respectively 23.6%, 41.0% and 30.6% of the WIS-AGA. These values, when based on juice weight, are comparable to the data reported in the literature (Table 11). The degree of esterification of the AGA present in the pectin fractions corresponds well with the values reported by Luh (1954a). As expected, the ammonium oxalate soluble pectin had a low degree of esterification, while the HCl soluble pectin was more highly esterified.
The ammonium oxalate soluble pectin was characterized by low quantities of arabinose and galactose; rhamnose was the major neutral sugar in this fraction. 7% of the AGA present in WIS and isolated from the oxalate extract had an apparent molecular weight of 40.000-80.000. The remaining oxalate soluble AGA was found in larger fragments with a wide MW distribution (Fig. 29b).
The HCl soluble pectin fraction contained 67% of the arabinose and 51% of the galactose present in the total WIS pectin. 15-16% of the WIS-AGA, solubilized by HCl, was present in a fragment with an estimated MW of $10^7$. This high molecular weight pectin fragment contained large amounts of neutral sugars, primarily rhamnose, arabinose and galactose (Fig. 29c,e). 10% of the AGA present in the WIS and solubilized by HCl was isolated as an intermediate sized fragment (figures 29c, d) with low amounts of arabinose and galactose (Table 35); in contrast to the oxalate soluble fragments with a low amount of arabinose and galactose the pectin in this fragment probably is more highly esterified.
All isolated pectin fragments contained small but significant amounts of xylose.
The NaOH soluble pectin fraction consisted of 44% protein but contained

only a small proportion of AGA. This result confirms data found by Gross (1984). This fraction was not characterized further because the pectin was saponified and partly degraded as a result of treatment with alkali.

Hemicelluloses from vegetables and fruits show a very diverse composition (Voragen et al. 1983, Siliha 1985, Holloway & Greig 1984). The tomato hemicellulose is characterized by high amounts of mannose (like mango and cherry), almost no fucose, and relatively low amounts of arabinose (in contrast to cherry, pineapple, apple and carrot) when compared to hemicelluloses of other sources. Ion exchange chromatography of tomato hemicellulose on DEAE-cellulose and analysis of glycosidic linkage composition of isolated fractions showed that 60% of the hemicellulose xylose is probably present as a 1,4-linked xylan with some branch-points at C-2 (fractions C,E and F, Table 37). 32% of the hemicellulose xylose might be present as xyloglucan (fractions A and D, Table 37). These results are in complete contrast to the results obtained with apple hemicellulose (Voragen et al. 1986 b). No indications were found for the presence of fucogalactoxyloglucans as reported by Voragen et al. (1986 b).
The fact that xylan co-elutes with AGA upon ion exchange chromatography (Table 37) and gel permeation chromatography (Fig. 31a, b), indicates a linkage between AGA and xylan. All the arabinose and the major part of the galactose was present as terminal residues. There were no indications for the presence of pectic arabinans in the hemicellulose; approximately 15% of the galactose residues were 1,4-linked and were possibly present as galactans. Only 10% of the arabinose found in hemicellulose was detected in the same fractions as the xylan; therefore the branching of xylan with arabinofuranoside was limited. 55% of the hemicellulose arabinose was found in the same fractions as the xyloglucan fragments. Part of the terminal arabinose molecules are connected to xylose residues, as is indicated by the composition of the dimer fraction obtained after fractionation of endo-glucanase treated hemicellulose on Bio-Gel P-2 (Fig. 32b).
The results also indicate the existence in tomato hemicellulose of a 1,4 bound (gluco)mannan with branch points at C-6 which may be linked to terminal galactose.

As a result of the incubation of tomato WIS with purified PL a high molecular weight highly branched pectin fragment (MW $\geq$ 80,000) was solubilized. This fragment contained 9% of the AGA, 26% of the arabinose,

16% of the galactose and in addition 5% of the xylose present in WIS. It is likely that this fragment forms part of the high MW HCl soluble pectin fragment rich in neutral sugars (pool I, Fig. 29c). Therefore, the pectin segments in this HCl soluble pectin fragment outside these branched regions consist of almost unbranched AGA chains with a high DE. These chains were degraded by PL. 40% of the WIS-AGA, solubilized as result of PL, was found in several fragments with an estimated MW of 10,000 - 40,000. These fragments only contained small amounts of arabinose and galactose (Table 41); since these fragments could not be further degraded by PL, their DE was probably low.

A pectin fraction with a MW of 40,000 - 80,000, containing 24% of the AGA present in WIS and low in arabinose and galactose content was isolated from the PG digest of tomato WIS. (Fig. 35a, b). With respect to molar sugar composition, MW and DE, this pectin fragment closely resembled a part of the HCl soluble pectin (Tables 35, 42, Figure 29d pool III); however, the total AGA quantity in the PG solubilized fragment was twice as high. 20% of the AGA present in the WIS was degraded to intermediate and small sized fragments as a result of PG action. It is probable that the major part of these degradation products originate from the ammonium oxalate soluble pectin fraction.

Simultaneous action of PE and PG released large amounts of arabinose and galactose; this is in contrast to the action of PG alone. The solubilized arabinose and galactose therefore originated from pectin fragments connected to the cell wall matrix through highly esterified pectin molecules. These results confirm the results obtained after PL action. The addition of PG to the residue of PL treated WIS and the addition of PL to the residue of PG treated WIS showed some degree of overlap in solubilized pectin. Both enzymes apparently solubilize a pectin fragment low in rhamnose, arabinose and galactose content. This view is further supported by the results of the gel permeation chromatography of the digest obtained after PL and PG action.

The fact that purified endo-galactanase released pectin fragments (MW 40,000 - 80,000, 21% of WIS-AGA) as well as fragments containing galactose and arabinose, indicate that arabinogalactans play a role in connecting pectic polymers to the cell wall matrix. These arabinogalactans, degraded by endo-galactanase, are not a part of the highly branched pectin fragment

released by PL. This is indicated by the observation that the amounts of arabinose and galactose solubilized by PL from the endo-galactanase treated WIS (Table 43) were similar to the amounts released by treatment of the WIS with PL (Table 39).

The exo-arabinanase, which is known to prefer highly branched arabinan as a substrate (Voragen et al. 1986c), solubilized 38% of the arabinose present in the WIS. Simultaneous addition of exo-arabinanase and PL indicated that the exo-arabinanase also acts on the highly branched PL solubilized fragment.

The addition of endo-glucanase and exo-glucanase to tomato WIS resulted in the solubilization of 23% of the WIS-AGA, which is of high MW. This implies the existence of a pectin fraction connected to cellulose or to hemicellulose polymers degradable by glucanases. It appeared that this fraction is degradable by PG but only partly by PL (Tables 39, 43); therefore the DE of this pectin must be relatively low. The points of attachment may be xylan polymers in the hemicellulose and/or the arabinan polymers which were found to exist in the cellulose fraction. In this connection it is interesting to note that xylose solubilization is enhanced by the simultaneous action of PE, PG, endo-glucanase and exo-glucanase, while arabinose solubilization is enhanced by simultaneous action of PL, endo-glucanase and exo-glucanase. The existence of arabinan chains as connections between pectin and the cell wall matrix is also indicated by the observation that a pure endo-arabinanase was able to release considerable amounts of AGA from tomato WIS (A.G.J. Voragen, personal communication).

Pectin solubilization was found to have no synergistic effect on cellulose degradation, which is in contrast to the data reported by Voragen et al. (1980) for the solubilization of cellulose from apple WIS and AIS.

## 5.5. *Conclusions*

The results of the studies on the polysaccharide fragments extracted chemically and enzymatically led to the following conclusions regarding the structural features of the tomato cell wall.

A large proportion of the pectin occurs as fragments with a low content of

arabinose and galactose side chains. These fragments are esterified in such a way that they are susceptible to both PG and PL action. PG degradation results in relatively highly esterified fragments with an apparent MW of 40,000-80,000; degradation with PL results in fragments with a lower DE and an apparent MW of 10,000-40,000.

Approximately 9% of the WIS-AGA forms part of a highly branched pectin segment containing 53% of the arabinose and 41% of the galactose present in the total pectin (respectively 26% and 16% of WIS-arabinose and WIS-galactose). These fragments are solely linked to the cell wall matrix through highly esterified AGA chains, which can only be degraded by PL. Endo-galactanase is not able to degrade the (arabino)galactan present in this fragment; exo-arabinanase is able to release arabinose molecules.

23% of the WIS-AGA is attached only to the hemicellulose and/or cellulose. The points of attachment may be the xylans present in the hemicellulose fraction or the arabinans which are found in the cellulose fraction. The DE of the pectin in these fragments is probably low.

21% of the WIS-AGA is present in the cell wall as fragments with an apparent MW of 40,000-80,000, connected to the cell wall matrix through (arabino)galactans. 7% of the WIS-AGA, also bound to (arabino)galactans, is of much higher apparent MW. Since this (arabino)galactan is degraded by endo-galactanase, its structure is not identical to the arabinogalactan present in the highly branched pectin fragments described above.

Xylans, arabinans and in particular (arabino)galactans are involved in the attachment of pectic substances to the cell wall matrix. A substantial amount of arabinose and galactose, however, is present in neutral sugar chains linked to pectic substances but not to other cell wall polymers. 40-50% of the arabinose present in WIS is found as terminally linked residues in hemicellulose polysaccharides. 35% of the WIS-arabinose is connected to galactans other than those found in the highly branched pectin fragment. An unknown part of the arabinose is probably present as slightly branched arabinan chains.

The tomato hemicellulose contains approximately 80% of the cell wall xylose. 60% of this amount is present in β-1,4-xylans. These xylans, branched with only small amounts of arabinose, are probably bound to pectin. 30% of the hemicellulose xylose was found to be present in fragments with a composition typical for xyloglucans.

The presence of glucomannans and a limited amount of galactan (± 7% of WIS-galactose) in the hemicellulose was also indicated. The major part of the galactose found in the hemicellulose (40-50% of WIS-galactose) was present as terminal residues.

# 6. CONTRIBUTION OF WIS CONSTITUENTS TO GROSS VISCOSITY

## 6.1. Introduction

The results presented in chapter 4 of this thesis show the important contribution of the WIS to the gross viscosity of tomato products. Gross viscosity at a specific WIS level was not influenced by serum viscosity, variety or concentration method.
In chapter 5 the composition and structural features of tomato WIS were discussed. The tomato varieties differed mainly in WIS content and in amounts of arabinose and galactose within this WIS. Tomato WIS was fractionated into 40-45% pectin, 25-30% hemicellulose and 30-35% cellulose. Moreover tomato WIS was found to contain approximately 20% protein, mainly present as part of the pectin fraction. The amount and composition of the pectin fractions was found to change as a result of processing.
The relative contribution of WIS constituents to the gross viscosity of tomato products remains unclear. In order to answer this question the effect of technical as well as purified, well characterized enzyme preparations on the gross viscosity of diluted tomato paste and a 10° Bx tomato concentrate was studied. The data found in the existing literature (Whittenberger & Nutting 1957, Smit & Nortje 1958, Foda & McCollum 1970, Brown & Stein 1977) are almost impossible to interpret because of the use of commercial, uncharacterized enzymes for the selective degradation of tomato constituents contributing to gross viscosity.

## 6.2. Methods

### 6.2.1. Enzyme treatment of diluted hot break paste

One part of variety H30 hot break paste (unpasteurized FHB juice concentrated in a R&C $T_{30}$ Anteo evaporator to 28-30° Bx, section 4.2.1) was thoroughly mixed with two parts distilled water. 1500 grams of this dilution (9.4°Bx, 1.83% WIS) was transferred into a water thermostated (40°C), double jacket reaction vessel as shown in Fig. 38. Enzymes were

added when the product had reached a temperature of 40°C (± ½ hr.). A sample of 150 grams was taken before the enzyme addition; other samples were taken after 5, 10, 20, 30, 60, 120, 180 and 300 minutes of enzyme action.

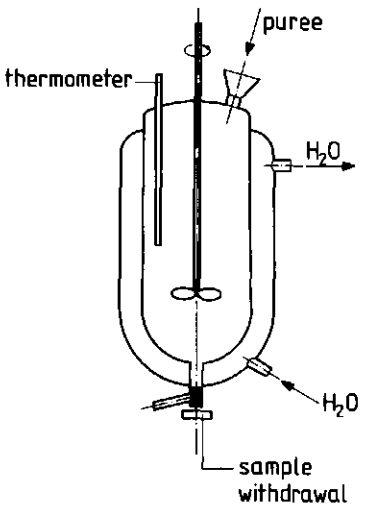



Fig. 38: Reaction vessel used for enzymatic treatment of diluted tomato paste.

All samples were immediately heated in boiling water for 15 minutes and then cooled to room temperature. The enzyme treated samples were analysed for gross viscosity, serum viscosity, °Bx, WIS content and WIS composition; the results were compared to the results of a blank treatment.
Changes in the appearance of the cell walls caused by enzyme action were determined by microscopic examination.
Table 44 shows the amounts of enzyme added to the substrate in weight % (on the basis of fresh weight and WIS weight) for the technical enzyme preparations and in units/kg fresh weight and units/g WIS for the purified enzymes. Table 45 shows some data on the enzyme activities of the technical enzyme preparations. Purified pectolytic enzymes did not show activities other than on their substrates.

Table 44: Enzyme additions to 2:1 diluted H30 paste.

| | fresh weight | | WIS | |
|---|---|---|---|---|
| | % w/w | U/kg | % w/w | U/g |
| Maxazym CL2000 | 0.125 | | 6.8 | |
| Rapidase C80 | 0.002 | | 0.11 | |
| Maxazym + Rapidase | 0.125 + 0.002 | | 6.8 + 0.11 | |
| Rohament P | 0.01 | | 0.55 | |
| Protease CGA 56470 | 0.1 | | 5.5 | |
| PG (Polygalacturonase) | | 36.7 | | 2.01 |
| PE (Pectinesterase) | | 18.5 | | 1.01 |
| PL (Pectinlyase) | | 25.8 | | 1.41 |
| PE + PG | | 18.5 + 36.7 | | 1.01 + 2.01 |

Maxazym CL2000: Gist-Brocades, Delft, The Netherlands

Rapidase C80: Gist-Brocades, Seclin, France

Rohament P: Röhm, Darmstadt, FRG

Protease CGA 56470: Ciba-Geigy AG, Basel, Switzerland

Table 45: Enzyme activities of technical enzyme preparations (U/g)

| enzyme activity | Rapidase C80 | Maxazym CL2000 | Rohament P | Protease CGA 56470 |
|---|---|---|---|---|
| Pectinesterase | 996 | - | - | - |
| Pectinlyase | 68 | - | 1.3 | - |
| Polygalacturonase | 421 | - | 760 | 0.44 |
| Exo-glucanase | - | 39 | 5.6 | |
| Endo-glucanase | 118 | 750 | 94 | |
| Cellobiase | 210 | 32 | | |
| Arabinanase | 44 | 0.5 | | |
| Galactanase | 38 | 0.4 | | |
| Protease[1] | + | - | | +++ |

1: +++ strong activity on gelatin layered film strip (5 hours 40°C),
+ activity, - no activity.

Variety H30 hot break tomato paste (28-30°Bx) was diluted in such a way that, after enzyme action, the WIS content was approximately 1%. The enzymes (Table 46) were added to the heated (40°C), diluted paste in the reaction vessel and the mixture was incubated for 7 hours. The enzyme treated sample was divided into 6 portions of 250 grams and stored overnight in a refrigerator. The next day the samples were "WIS-concentrated"; 0, 14%, 25%, 35%, 41% and 48% of the weight was removed as clear serum after centrifugation for 30 minutes at 24,500 x g. The particles were resuspended in the remaining serum and the gross viscosity was measured with both the Haake rotovisco and the Bostwick consistometer. WIS was prepared in duplicate from a non-centrifuged, enzyme treated sample. The WIS amounts in the "WIS-concentrated" samples were calculated from this determined value.

Table 46: Dilution of tomato paste and amounts of enzyme added to the diluted paste samples.

| | | | enzyme addition | |
|---|---|---|---|---|
| enzyme treatment | grams paste | grams water | % w/w | units |
| Maxazym CL2000 | 400 | 1200 | 0.125 | |
| | 350 | 1200 | 0.01 | |
| Rapidase C80 | 350 | 1200 | 0.002 | |
| Maxazym + Rapidase | 400 | 1200 | 0.01 + 0.002 | |
| Rohament P | 350 | 1200 | 0.01 | |
| Protease CGA 56470 | 400 | 1200 | 0.10 | |
| PE | 300 | 1200 | | 36.8 |
| PG | 350 | 1200 | | 11.2 |
| PE + PG | 350 | 1200 | | 36.8 + 11.2 |
| PL | 350 | 1200 | | 38.6 |
| Blank | 300 | 1200 | - | - |

The WIS was analysed for AGA, degree of esterification of the AGA, protein, cellulose and neutral sugar content. The exact procedure for paste dilution and enzyme addition is shown in Table 46.

### *6.2.2. Enzyme treatment of a 10°Bx tomato juice concentrate*

1.5 kg. of a 10°Bx concentrate (1.99% WIS) of a variety 318 hot break juice (hot break temperature 93°C, finisher screen size 1.5 mm) was divided into portions of 100 grams. 0.1% sodium benzoate was added as a preservative. The samples were heated to 40°C in a water bath before the appropriate amounts of enzyme (Table 47) were added.

Table 47: Enzyme additions to 10°Bx tomato concentrate (on basis of WIS).

| | enzyme addition | |
|---|---|---|
| enzyme treatment | % w/w | U/g |
| Maxazym CL2000 | 6.44 | |
| Rapidase C80 | 0.10 | |
| Maxazym + Rapidase | 6.44 + 0.10 | |
| Rohament P | 0.52 | |
| Ultrazym M10* | 0.13 | |
| Protease CGA 56470 | 4.26 | |
| PG | | 1.91 |
| PE | | 1.03 |
| PE + PG | | 0.85 + 0.72 |
| PL | | 1.29 |

* Ultrazym M10 (Novo Ferment AG, Basel, Switzerland) is comparable to Rohament P but approximately 4 times as concentrated.

Exactly 2 minutes after adding the enzyme a continuous gross viscosity measurement was started in the Haake rotovisco using rotary bob MVIII at a speed of 54 rpm and at a temperature of 40°C. The measurement was stopped after 5 hours; the enzyme treated sample was stored overnight in a refrigerator before °Bx, serum viscosity and % WIS were determined. The WIS was analysed for AGA, DE of AGA, cellulose, protein and neutral sugar content.

## 6.3. *Results and discussion*

### 6.3.1. *Effect of enzyme treatment on gross viscosity and composition of diluted hot break paste*

Table 48 shows data on gross and serum viscosity and composition of the 2:1 diluted tomato paste used as a substrate for the enzymes. The data are an average of 11 individual determinations, one for each enzyme treatment. The gross viscosity of this diluted paste sample is only 70% of the gross viscosity of a tomato juice after concentration to 1.83% WIS (compare Fig. 24, chapter 4). The phenomenon of a loss in gross viscosity as a result of concentration and subsequent dilution ("dilution-loss") will be discussed extensively in chapter 7.

Table 48: Gross viscosity, serum viscosity and composition of diluted tomato paste of variety H30.

| | | |
|---|---|---|
| $\eta_{app}$ | (mPa.s) | 423.1 ± 25.5 |
| $\eta_{serum}$ | (mPa.s) | 8.2 ± 0.3 |
| °Bx | | 9.4 ± 0.1 |
| WIS | (mg/100 g) | 1830 ± 33 |
| ACA | (mg/100 g) | 230 ± 14 |
| DE | | 34 ± 3 |
| Protein | (mg/100 g) | 379 ± 12 |
| Cellulose | (mg/100 g) | 578 ± 26 |
| Rhamnose | (mg/100 g) | 11.0 ± 1.0 |
| Arabinose | (mg/100 g) | 22.9 ± 1.4 |
| Xylose | (mg/100 g) | 63.9 ± 5.6 |
| Mannose | (mg/100 g) | 51.5 ± 4.1 |
| Galactose | (mg/100 g) | 52.3 ± 3.6 |
| Glucose | (mg/100 g) | 536.8 ± 40.0 |

Figure 39 shows the effects of enzyme addition on the gross viscosity of the diluted tomato paste. It is apparent from these figures that all the

enzymes or combinations of enzymes, except PE, are able to decrease gross viscosity.

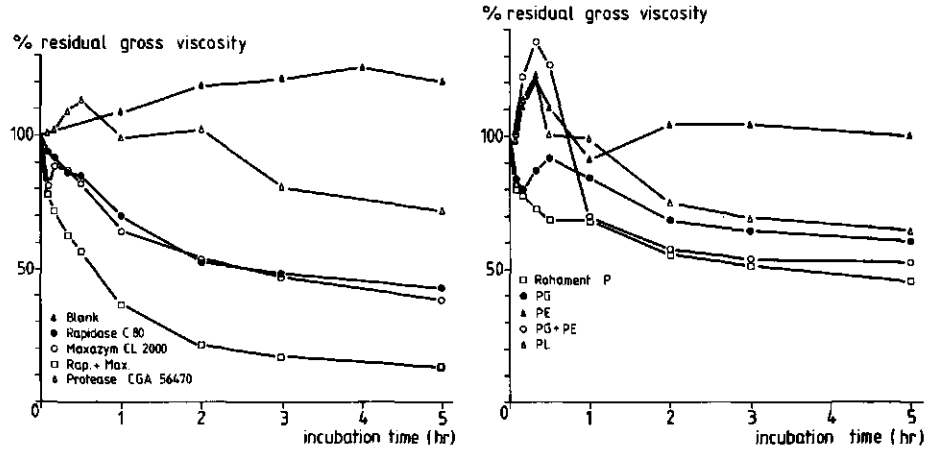



Fig. 39: Change in gross viscosity (Haake) of diluted tomato paste as a result of treatment with enzymes.

The largest decrease in gross viscosity (86%) is obtained with a combination of a technical pectolytic (Rapidase C80) and cellulolytic (Maxazym CL2000) enzyme preparation. The role of pectin in determining gross viscosity is indicated by the action of technical and purified pectolytic enzyme preparations. A reduction of 35% to 60% in gross viscosity can be obtained by degrading certain parts of the WIS pectin. The role of cellulose in determining gross viscosity must not be underestimated. Fig. 39 clearly shows that degradation of cellulose may result in a gross viscosity loss as great as that obtained with the pectolytic enzyme Rapidase C80. The smallest effects on gross viscosity were observed with PE and protease. PE deesterifies highly esterified pectin without affecting the glycosidic bonds in the pectin chain. Therefore one would not expect a large change in gross viscosity as a result of PE enzyme action. Although the WIS contains more than 20% protein (1.5 times the AGA content) the results do not indicate that protein makes an important contribution to gross viscosity.

Some interesting initial effects are observed when pectolytic enzymes are added to the diluted tomato paste (Fig. 39). A sharp initial decrease in gross viscosity is found after the addition of PG and Rohament P; the addition of PL, PE and PE + PG results in an initial increase in gross viscosity which reaches a maximum 20 minutes after the addition of the enzyme. After the initial decrease, the gross viscosity of PG treated diluted tomato paste increases to a maximum after 30 minutes of enzyme incubation.

The chemical changes in WIS accompanying the sharp initial decrease in gross viscosity caused by PG and Rohament P action are presented in Table 49.

Table 49: Decrease in gross viscosity, in WIS content and in amount of WIS constituents after 10 min. enzyme action (in %).

| | $\eta_{app}$ | WIS | AGA | DE[1] | Protein | Cellulose | Rham. | Ara. | Xyl. | Man. | Gal. | Gluc. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rohament P | 22 | 7 | 39 | 48 | n.s. | n.s. | 30 | 17 | n.s. | n.s. | 19 | n.s. |
| PG | 20 | 2 | 27 | 49 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | 13 | n.s. |

1: calculated DE of solubilized AGA.

n.s. = not significant.

30-40% of the AGA present in WIS is rapidly solubilized by the action of PG (purified or in Rohament P), resulting in a gross viscosity loss of up to 22%. The loss in WIS is completely covered by the solubilization of AGA with a degree of esterification of 48-49%. Results presented in the preceeding chapter showed that incubation of WIS with a purified PG resulted in the solubilization of pectin fragments with an apparent MW of 40,000-80,000, containing 24% of the AGA present in WIS (DE=58). The solubilization of these pectin fragments obviously caused the rapid decrease in gross viscosity of the diluted tomato paste found after the addition of PG or Rohament P. The addition of PE + PG or PL resulted only in the solubilization of 10-12% of AGA in the first 10 minutes of enzyme action.

Table 50 shows the chemical changes in the WIS accompanying the initial increase in gross viscosity as a result of the addition of PL, PE and PE + PG. The results for the addition of PG are also included because this enzyme causes a gross viscosity increase after the initial decrease. The gain in gross viscosity as measured after 20 minutes of enzyme action may be as high as 35% (PE + PG). It was observed that gross viscosity increased simultaneously with WIS, xylose, mannose, glucose and cellulose as determined by the Updegraff method.

Table 50: Changes in gross viscosity, in WIS content and in WIS composition after 20 min. (PE, PE + PG, PL) and 30 min. (PG) enzyme action (in %).

| | $\eta_{app}$ | WIS | AGA | Protein | Cellulose | Rham. | Ara. | Xyl. | Man. | Gal. | Gluc. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PE | +22 | +11 | + 8 | + 9 | +18 | +15 | +18 | +11 | +26 | n.s. | +12 |
| PE + PG | +35 | +11 | -13 | + 9 | +21 | n.s. | n.s. | +25 | +11 | +19 | +22 |
| PL | +23 | + 6 | -13 | + 6 | +11 | n.s. | n.s. | +15 | +19 | n.s. | +14 |
| PG | - 8* | + 2 | -26 | n.s. | + 6 | n.s. | n.s. | +15 | +14 | n.s. | + 6 |

* was -20% after 10 min. incubation time.

n.s.: not significant.

Moreover, the amounts of solubilized AGA did not increase between 5 and 20 minutes of enzyme incubation with PE, PE + PG and PL; no increases in solubilized AGA were found between 5 and 60 minutes of incubation with PG. The increase in WIS content may be attributed to the formation of aggregates as a result of the heat treatment necessary for the inactivation of the enzymes. In another experiment it was observed that the heating of PE + PG treated serum resulted in the formation of a precipitate; no precipitation was found after the heating of untreated serum.
The increase in gross viscosity may be caused by a partial negation of the "dilution-loss" (see 7.3.3); no increase in gross viscosity was detected when a 10° Bx tomato concentrate was treated with enzymes (see 6.3.2., Fig. 42).

After 5 hours of enzyme action both Rohament P and PG had degraded large parts of the WIS pectin, causing a decrease in gross viscosity of

40-50% (Table 51). No degradation of other cell wall polymers was detected.

Table 51: Decrease in gross viscosity, in WIS content and in amount of WIS constituents as a result of enzyme action for 5 hours on a 2:1 diluted tomato paste (in %).

| Enzyme | $\eta_{app}$ | WIS | AGA | DE[3] | Rham. | Ara. | Gal. | Cellulose | Xyl. | Man. | Protein |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rohament P | 54 | 15 | 61 | 4 | 57 | 21 | 26 | 5 | n.s. | n.s. | n.s. |
| PG | 40 | 12 | 43 | 20 | 22 | 20 | 20 | 5 | n.s. | n.s. | n.s. |
| PE[1] | n.s. | n.s. | n.s. | 25 | n.s. | 11 | 16 | n.s. | n.s. | n.s. | 7 |
| PE + PG | 47 | 12 | 57 | 0 | 47 | 35 | 22 | 5 | n.s. | n.s. | n.s. |
| PL | 35 | 12 | 42 | 8 | 39 | 29 | 23 | 8 | n.s. | n.s. | n.s. |
| Rapidase C80 | 57 | 18 | 62 | 0 | 68 | 43 | 38 | n.s. | 11 | n.s. | 5 |
| Maxazym CL2000 | 61 | 24 | 11 | 36 | 24 | 35 | 21 | 44 | 52 | 53 | n.s. |
| Rap. + Max.[2] | 86 | 39 | 64 | 2 | 73 | 62 | 47 | 39 | 48 | 36 | 11 |
| Protease | 25 | 16 | 18 | 32 | 29 | 34 | 11 | 8 | 15 | 8 | 30 |

1: PE reduced DE of soluble AGA from 51 to 18.

2: Determination of neutral sugar content after 180 min. instead of 300 min.

3: DE of unsolubilized AGA

n.s. not significant

The differences found between the effects of Rohament P and purified PG are probably due to a difference in the amount of units of PG added to the diluted tomato paste. Both enzymes released the major part of the total solubilized AGA in the initial stage of the reaction; smaller amounts were released as result of the subsequent period of enzyme action (Table 52). The DE of the AGA solubilized after the initial stage is higher than that of the pectin solubilized initially. From the results it can be concluded that PG solubilizes pectin with an overall DE of 50-60% (DE of total WIS pectin = 34%). The solubilization of this type of pectin greatly influences the gross viscosity of the diluted tomato paste.

Although the total effect of PL on gross viscosity is comparable to the effects of PG and Rohament P, the mechanism of PL action is quite

different. PL solubilizes 10% of the AGA with a DE of 94 in the first 10 minutes of enzyme incubation (Table 52).

Table 52: Effect of pectolytic enzymes on pectin degradation and gross viscosity loss (in %).

| | AGA decrease | | DE solubilized AGA[3] | | gross viscosity loss | |
|---|---|---|---|---|---|---|
| | 0-10 min. | 10-300 min. | 0-10 min. | 10-300 min. | 0-10 min. | 10-300 min. |
| Rohament P | 38 | 23 | 48 | 66 | 22 | 32 |
| PG | 27 | 17 | 49 | 52[1] | 20 | 20 |
| PL | 10 | 31 | 94 | 50[2] | increase 14 | 35 |

1 DE increases to 64 after 1 hour of enzyme incubation.

2 DE decreases to 38 after 1 hour of enzyme incubation.

3 calculated.

This did not result in a decrease in gross viscosity, presumably due to the simultaneous increase in WIS (Table 50) and to the partial negation of the "dilution-loss". After 1 hour of enzyme action, when 21% of the AGA (average DE=83) had been solubilized, the gross viscosity returned to the same level as found at the start of the incubation. Between the 2nd and the 5th hour of enzyme action another 21% of the AGA (average DE=38) was solubilized; the total AGA solubilization of 42% (average DE=62) finally resulted in a gross viscosity loss of 35%. As will be shown in 6.3.2., the effect of PL on gross viscosity depends on the DE of the total WIS pectin.

In chapter 5 pectin fragments susceptible to degradation by both PL and PG were identified. The results presented in this chapter indicate that these fragments contribute to a great extent to gross viscosity. The results obtained by the simultaneous addition of PE and PG and by the addition of Rapidase C80 confirm the important contribution of the higher esterified parts in the WIS pectin to gross viscosity. The contribution of the lower esterified parts in the WIS pectin, however, remains unclear. From the results obtained after the addition of Rohament P it can be concluded that approximately 40% of the WIS pectin has a low degree of

esterification (Table 51). Unfortunately the added enzymes were not able to solubilize this type of pectin under the reaction conditions used. The fact that even PG is unable to solubilize this 40% of low esterified pectin may be attributed to a high degree of acetylation of this pectin or to an inaccessibility of this pectin for the enzymes.

The gross viscosity decrease as a result of the addition of Maxazym CL2000 is comparable to the decrease obtained with Rapidase C80 (Table 51). In order to reach this decrease, Maxazym CL2000 needs to degrade 24% of the WIS compared to 18% for Rapidase C80. Cellulose degradation accounts for almost 60% of the WIS solubilization found after Maxazym CL2000 incubation. This enzyme degraded not only cellulose but also hemicellulose (xylose, mannose) and parts of the WIS pectin connected to hemicellulose and/or cellulose. In the first hour of enzyme incubation no solubilization of pectin was found. Cellulose, however, solubilized for 20% (= 6.3% of WIS), xylose and mannose for 25% (= 1.4% of WIS), and gross viscosity decreased by 36%. It seems likely that the decrease in gross viscosity as a result of Maxazym CL2000 action is mainly caused by the degradation of cellulose.

The combined action of Rapidase C80 and Maxazym CL2000 greatly decreased gross viscosity. The cell structure was severely attacked as was shown by microscopic examination (Fig. 40). Rapidase C80 alone, like the other pectolytic enzymes, had no distinct effect on the microscopic appearence of the cells; Maxazym CL2000 treated cells had thinner walls when compared to untreated cells, but were unbroken.

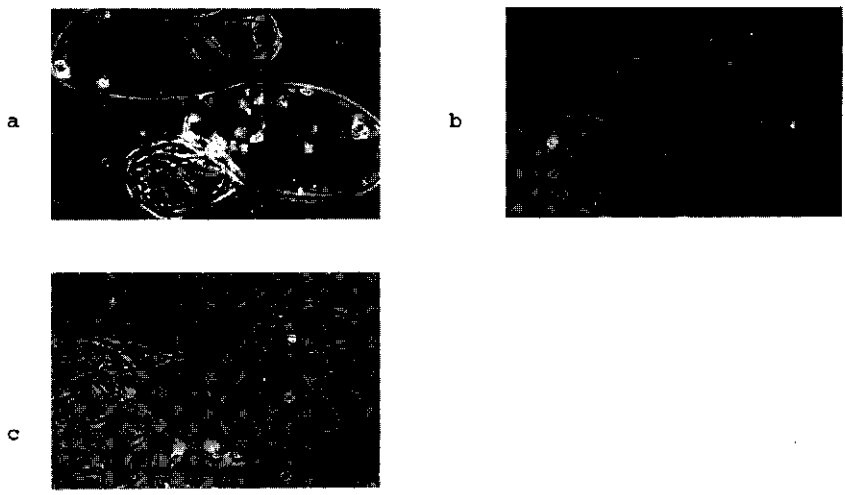



Fig. 40: Effect of enzymes on the structure of tomato cells.
a: control, b: Maxazym CL2000, c: Rapidase C80 + Maxazym CL2000 (magnification 61.4x).

The solubilization of 30% of the protein as a result of the action of protease resulted in a gross viscosity loss of 25% (Table 51). It is, however, questionable whether this gross viscosity loss is caused by protein solubilization or by the solubilization of 18% AGA and 8% cellulose. The role of protein as a contributor to gross viscosity is more clearly shown in Fig. 41.

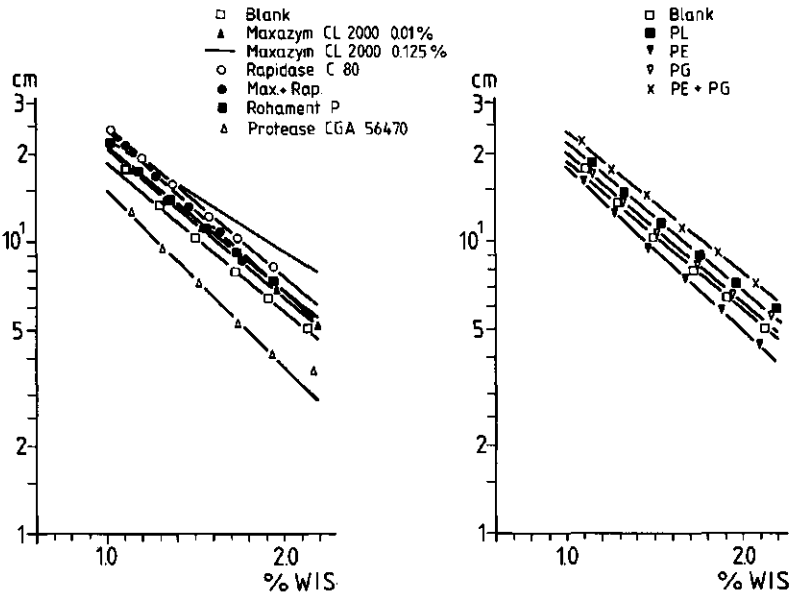



Fig. 41: "WIS-concentration" curves (Bostwick) of enzyme treated, diluted tomato paste.

This figure shows the "WIS-concentration" curves of samples of diluted tomato paste incubated with enzymes for 7 hours. The slope and intercept of these straight lines indicate the potential of enzymatically modified WIS to "build" gross viscosity (WIS-quality). Similar curves were obtained after the measurement of gross viscosity with the Haake rotovisco instead of the Bostwick consistometer.

The incubation of the diluted paste with protease resulted in the solubilization of 50% of the protein, 22% of the AGA and 8% of the cellulose present in WIS. The solubilized protein obviously does not contribute to gross viscosity.

Protease treated diluted paste concentrated to a specific WIS content shows increased gross viscosity when compared to untreated diluted paste at the same WIS concentration. It must be realized that at this WIS concentration the protease treated paste contains more tomato cells and is therefore enriched in the constituents determining gross viscosity.
An increase in gross viscosity at a specific WIS amount was also found when the diluted paste was incubated with PE. Due to the increase in calcium reactive COOH-groups, the PE treated diluted paste transformed into a gel upon storage.
The poorest WIS-quality at 1% WIS was obtained after treatment of the diluted paste with Rapidase C80, or with amounts of purified PE and PG comparable to the amounts present in Rapidase C80. (Fig. 41).
The incubation of the diluted paste with 0.125% Maxazym CL2000 resulted in the solubilization of 78% of the cellulose, 30% of the AGA and 11% of the protein present in the WIS; moreover 80-90% of the WIS-xylose and WIS-mannose was degraded. The WIS concentration curve of this enzyme treated, diluted paste shows a completely different slope than the other curves (Fig. 41). At the lower WIS amounts (± 1%) the loss in cellulose is apparently compensated for by relatively high amounts of tomato cells and therefore of AGA. At the higher WIS amounts the decreased rigidity of the cell walls, caused by the loss in cellulose, seems to become increasingly important in maintaining gross viscosity (Table 53). Shomer (1984) showed that a partial degradation of the microfibrillar cellulose structure by 0.2% of an unspecified cellulase resulted in a collapse of the cell wall precipitate under gravity stress. This loss in ability to withstand collapse obviously also affects the gross viscosity.
The incubation of diluted tomato paste with Rapidase C80, PE + PG and Rohament P showed that approximately 40% of the WIS-AGA cannot be solubilized by these enzymes (Table 51). Moreover, results obtained after the addition of Rohament P (no PE activity) indicated that this unsolubilized pectin is almost unesterified. It can be assumed that PL and PG will also be unable to solubilize this type of pectin. Therefore it can be calculated from the results shown in Table 53, that the WIS in the PG treated tomato paste contains a certain amount of highly esterified pectin; this in contrast to the WIS of PL treated tomato paste. This amount of highly esterified pectin (44 mg/100 g) seems to be the reason for the

higher gross viscosity of the PG treated sample when compared to the PL treated sample (Table 53).

Table 53: Gross viscosity and composition (mg/100 g) of enzyme treated diluted tomato paste at 2% WIS.

| Enzyme | Bostwick cm | AGA | DE | Cellulose | Protein |
|---|---|---|---|---|---|
| Maxazym CL2000 0.125% | 9.4 | 357 | 29 | 263 | 644 |
| PE + PG | 7.7 | 114 | 0 | 743 | 509 |
| Rapidase C80 | 7.6 | 113 | 0 | 717 | 471 |
| Max. 0.01% + Rap. | 6.8 | 131 | 0 | 709 | 526 |
| Rohament P | 6.8 | 110 | 0 | 783 | 480 |
| PL | 6.7 | 147* | 4* | 718 | 503 |
| Maxazym CL2000 0.01% | 6.5 | 264 | 35 | 632 | 454 |
| PG | 6.1 | 143** | 19** | 674 | 470 |
| Blank | 5.8 | 286 | 32 | 660 | 404 |
| PE | 4.9 | 259 | 15 | 628 | 450 |
| Protease | 3.7 | 274 | 34 | 745 | 254 |

* probably 98 mg with DE 0 and 49 mg with DE 12 (calculated from Rohament P)

** probably 99 mg with DE 0 and 44 mg with DE 64 (calculated from Rohament P)

It must be realized that the differences in WIS composition as caused by the addition of enzymes are much larger than the differences which occur naturally in tomato WIS.

Differences in gross viscosity are caused rather by differences in WIS amount (amount of tomato cells) than by the small differences in WIS composition. The small differences in gross viscosity found at a specific WIS amount may be explained by a difference in content and characteristics of AGA or by a difference in rigidity of the cell walls.

### 6.3.2. *Effect of enzyme treatment on gross viscosity and composition of a 10°Bx tomato juice concentrate*

A 10°Bx tomato juice concentrate was also subjected to enzyme treatment. Its composition (Table 54) is comparable to the composition of the diluted paste (Table 48), except for cellulose content and DE of the pectin. A major difference between the two substrates is the level of the gross viscosity. The higher gross viscosity of the 10°Bx concentrate can be attributed to the absence of a "dilution-loss".

Table 54: Gross viscosity, serum viscosity and composition of a 10°Bx concentrate of variety 318.

| | | |
|---|---|---|
| $\eta_{app}$ | (mPa.s) | 614.7 |
| $\eta_{serum}$ | (mPa.s) | 8.6 |
| °Bx | | 9.9 |
| WIS | (mg/100 g) | 1990 |
| AGA | (mg/100 g) | 245 |
| DE | | 44 |
| Protein | (mg/100 g) | 373 |
| Cellulose | (mg/100 g) | 663 |
| Rhamnose | (mg/100 g) | 12.7 |
| Arabinose | (mg/100 g) | 27.5 |
| Xylose | (mg/100 g) | 77.0 |
| Mannose | (mg/100 g) | 51.7 |
| Galactose | (mg/100 g) | 66.5 |
| Glucose | (mg/100 g) | 571.5 |

Changes in gross viscosity and in the composition of the tomato concentrate as a result of incubation with the enzymes are presented in Table 55 and Fig. 42. The curves shown in Fig. 42 are corrected for a blank treatment. In general, the addition of enzymes to the 10°Bx tomato concentrate led to the same final results as obtained by the addition of enzymes to the diluted paste. A sharp initial decrease in gross viscosity was again found after incubation with Rohament P, PG and also with

Ultrazym M10. The initial decreases as a result of Rapidase C80, PL and PE + PG action were smaller; the final effects, however, were much greater.

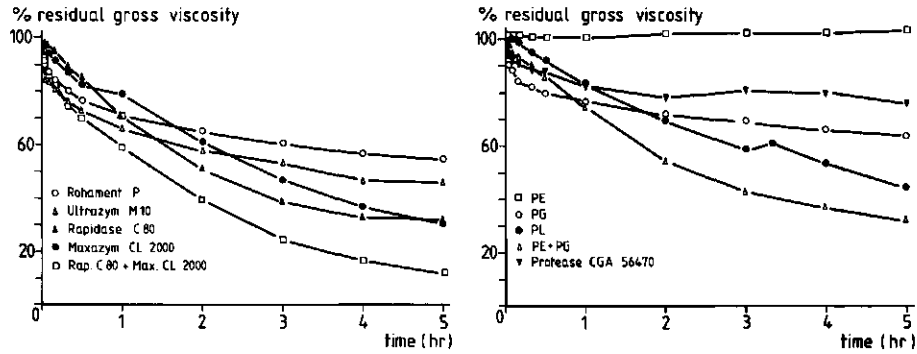



Fig. 42: Changes in gross viscosity (Haake) of a 10°Bx tomato concentrate as a result of treatment with enzymes.

Table 55: Reduction in gross viscosity, in WIS content and in amount of WIS constituents as a result of enzyme action for 5 hours on a 10°Bx tomato concentrate (in %).

| | $\eta_{app}$ | WIS | AGA | DE[1] | Rham. | Ara. | Gal. | Cellulose | Xyl. | Man. | Protein |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rohament P | 45 | 15 | 60 | 20 | 52 | 32 | 29 | n.s. | n.s. | +22 | 7 |
| Ultrazym M10 | 54 | 21 | 70 | 6 | 72 | 51 | 38 | n.s. | n.s. | +16 | 9 |
| PG | 36 | 13 | 47 | 35 | 21 | 17 | 22 | 9 | n.s. | n.s. | 6 |
| PE | +3 | +3 | +6 | 25 | n.s. | n.s. | n.s. | 6 | n.s. | n.s. | 5 |
| PE + PG | 67 | 14 | 63 | 5 | 44 | 35 | 31 | 5 | n.s. | n.s. | n.s. |
| PL | 54 | 14 | 53 | 18 | 36 | 30 | 25 | 8 | n.s. | n.s. | 5 |
| Rapidase C80 | 68 | 20 | 70 | 0 | 80 | 53 | 46 | 5 | n.s. | +16 | 6 |
| Maxazym CL 2000 | 68 | 36 | 25 | 43 | 25 | 29 | 32 | 67 | 71 | 76 | 8 |
| Rap. + Max. | 87 | 45 | 70 | 0 | 89 | 70 | 68 | 62 | 62 | 61 | 10 |
| Protease CGA 5647 | 24 | 12 | 17 | 42 | 11 | 18 | 7 | 4 | 9 | n.s. | 35 |

1: DE of unsolubilized AGA

n.s.: not significant

The largest gross viscosity loss was obtained with the combination Rapidase C80 and Maxazym CL2000; the smallest loss in gross viscosity with Protease CGA 56470. The addition of PE to the tomato concentrate had no influence on gross viscosity.

Some interesting differences in the effect of enzymes on the gross viscosity of the two substrates were noticed. In contrast to results obtained for the diluted paste, no increase in gross viscosity was found after the addition of PL, PE and PE + PG to the tomato concentrate (Fig. 42). This difference may be caused by the absence of a "dilution-loss" in the 10°Bx tomato concentrate (see 6.3.1., 7.3.3.).

Pectolytic enzymes capable of degrading esterified pectin (PL, PE + PG, Rapidase C80) have a greater influence on gross viscosity of the 10°Bx concentrate (DE of the AGA = 44), than on gross viscosity of the diluted paste (DE of the AGA = 34). PL, for example, solubilized 53% of the WIS-AGA present in the 10°Bx concentrate, with an overall DE of 68%, resulting in a gross viscosity loss of 54%. 42% of WIS AGA (DE=62) was solubilized from the diluted paste, resulting in a gross viscosity loss of 35%.

## 6.4. *Conclusions*

All results, irrespective of the tomato product used, point to an important role for the more highly esterified parts of the WIS pectin in contributing to gross viscosity. It is likely that these pectins are identical to the pectins susceptible to degradation by both PL and PG as described in chapter 5. The degradation of these pectins by PG results in the immediate solubilization of fragments with a high MW and a high DE, giving rise to a rapid decrease in gross viscosity. PL initiates the degradation of these highly esterified fragments; pectin solubilizes more slowly and therefore gross viscosity decreases gradually.

The importance of the low esterified pectin remains unclear; none of the enzymes was capable of selectively solubilizing this type of pectin. The same holds for the contribution of hemicellulose polymers to gross viscosity. The role of the protein present in the WIS is probably negligible. Cellulose seems to be an important WIS constituent with regard to gross viscosity. Its relative contribution to gross viscosity is

smaller than the contribution of the esterified pectin; solubilization of 67% of the cellulose (22.3% of the WIS) resulted in the same gross viscosity loss as solubilization of 70% of the AGA (8.6% of the WIS). The rigidity of the cell walls, caused by the presence of cellulosic structures, seems to influence gross viscosity at higher WIS concentrations.

Natural differences in WIS composition, which are much smaller than the differences brought about by the addition of enzymes, cannot cause large differences in gross viscosity. Gross viscosity is primarily determined by the amount of WIS (amount of tomato cells). The presence of large amounts of high ester pectin in the WIS may have some influence on gross viscosity at a specific WIS amount. The rigidity of the cell walls may influence gross viscosity at higher WIS concentrations.

# 7. *INFLUENCE OF PROCESSING FACTORS ON THE WIS CONTENT, WIS COMPOSITION AND GROSS VISCOSITY OF TOMATO JUICE AND CONCENTRATES*

## 7.1 *Introduction*

The gross viscosity of tomato juice and concentrates is determined mainly by its WIS content; serum viscosity does not contribute to gross viscosity (chapter 4). The differences observed between varieties were explained in terms of a different WIS content; large differences in WIS composition were not found (chapter 5). Data shown in chapter 6 indicate the importance of more highly esterified parts of the WIS pectin and of cellulose as contributors to gross viscosity. Cellulosic structures also determine the rigidity of the cell walls. This rigidity seems to influence gross viscosity at higher WIS concentrations. The amount and composition of the pectin fractions were found to change as a result of processing (chapter 5).
This chapter deals with the influence of processing factors such as hot break temperature, finishing operation, concentration and pasteurization on the WIS characteristics and gross viscosity of tomato juice and concentrates.

## 7.2. *Methods*

### 7.2.1. *Production scale equipment used to produce samples for studying the effects of processing conditions on gross viscosity*

The samples used to study the influence of processing conditions on the gross viscosity of tomato juice and concentrates were produced mainly in a Portuguese factory in the region of Lisbon. Samples prepared at different hot break temperatures were produced both in this factory as well as in an Italian factory in the region of Parma. The equipment used in these two factories is listed below.

*Portugal*

hot break: Rossi & Catelli 1000 L recirculating chop/scalder. (see section 4.2.1.).

finishing: Rossi & Catelli three stage paddle finisher 133 b-14. (capacity 14 tons/hr.).

evaporation: Rossi & Catelli double effect $T_{30}$ D.F.F. Anteo evaporator. (see section 4.2.2.).

*Italy*

hot break: Rossi & Catelli "Eldorado" scalder (Fig. 44). This scalder is basically composed of a vacuum tank and a pressurized hot section tank coupled to a tubular heat exchanger. Due to the presence of the pressurized section, hot break temperatures in excess of 100°C can be obtained. The capacity of this scalder is 35 tons/hr.

finishing: Rossi & Catelli two stage "Butterfly" finisher, maximum capacity 20 tons/hr.

evaporation: Rossi & Catelli double effect T60 Califfo evaporator. This evaporator produces ± 5 tons 23°Bx paste/hr. at an evaporation rate of 22,000 kg water/hr. Product temperatures were 68°C (54 cm Hg vacuum) in the first effect and 46°C (68.5 cm Hg vacuum) in the second effect.

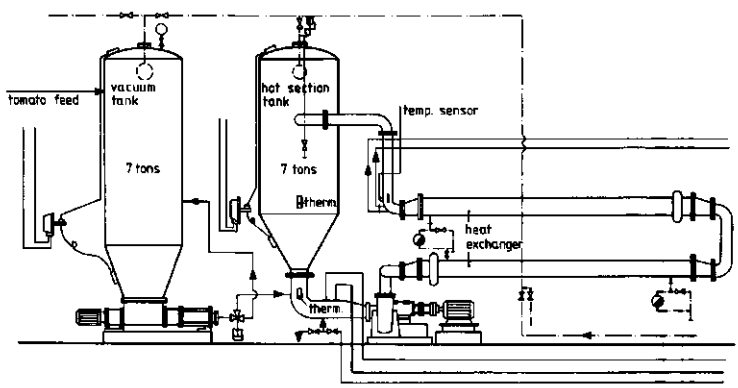



Fig. 44: The Rossi & Catelli "Eldorado" scalder.

### 7.2.2. *Production of tomato juice and paste at various hot break temperatures*

Tomato juices prepared at hot break temperatures ranging from 90°C to 103°C were produced in Portugal in the 1983 and 1984 seasons. The break temperatures were recorded at the outlet of the tubular heater and therefore represent final product temperatures. The temperatures measured in the chopper were, in general, 4-6°C lower.
Screen openings used in the pulper, refiner and superrefiner units of the finisher were respectively 1.0 mm, 1.0 mm and 0.8 mm. Juice samples were heat processed for 45 min. at 100°C.

In 1984 and 1985 tomato paste was produced in Italy from tomato juice prepared at hot break temperatures ranging from 90°C to 115°C (final product temperature). In order to minimize the residence time of the broken tomatoes in the vacuum chamber of the Eldorado scalder (enzyme action) the level of tomatoes in this tank was kept at 1-2 tons.
Screen openings used in the Butterfly pulper/finisher were respectively 1.2 mm - 0.6 mm in 1984 and 0.8 mm - 0.8 mm in 1985. The tomato juice was concentrated to a level of 23°Bx, sterilized for 2 min. 15 sec. at 109°C and then packed aseptically in metalized film bags. The sampling of the tomato paste was started no earlier than 4 hours after a change in hot break temperature. Each experiment was continued for at least 10 hours.

### 7.2.3. *Production of tomato juice and paste using different finishers and finisher conditions*

In order to study the effects of type of finisher, finishing temperature and finisher screen opening on gross viscosity, the following samples were prepared.
Tomatoes of the variety H30 were crushed at hot break temperatures of 90°C and 103°C. A part of the crushed tomatoes was processed using the three stage paddle finisher (1.0 mm - 1.0 mm - 0.8 mm screens). The juice was canned and heat processed for 45 min. at 100°C. Another part of the crushed tomatoes was finished, either at room temperature or after reheating to 90°C, using a laboratory Bauknecht KU 2-1 tapered screw juice extractor equipped with a 0.9 mm screen (batch size 2.4 kg). These juices were not heat processed.

Tomato juice was prepared from tomatoes of the variety H30 at a hot break temperature of 103°C. Different combinations of screens were used to finish the crushed tomatoes. (Table 57).

Table 57: Screen combinations used for finishing hot break crushed tomatoes.

| pulper | refiner | superrefiner |
|---|---|---|
| 1.5 mm | 1.5 mm | - |
| 1.5 mm | 1.5 mm | 1.0 mm |
| 1.5 mm | 1.5 mm | 0.8 mm |

The juice was concentrated to 29°Bx and packed aseptically in metalized bags.

5.5 kg of fresh tomatoes of the variety H30 were heated (20 min. 120°C) in closed sterilization bags in a retort to a heart temperature of 90°C. The hot tomatoes were finished using the tapered screw juice extractor equipped with a 0.5 mm or a 1.5 mm screen. The juices were not heat processed.

### *7.2.4. Concentration of tomato juice*

#### *Preparation of juice samples*

All the juices used to study the influence of the concentration process on the gross viscosity of concentrates and diluted concentrates were produced in Portugal at a hot break temperature of 103°C. The pulper, refiner and superrefiner screen openings used to finish the crushed tomatoes were respectively 1.0 mm, 1.0 mm and 0.8 mm. The juices were canned and heat processed for 45 min. at 100 °C, unless they were used directly for concentration in the T30 double effect evaporator, or for separation in a decanter centrifuge.

*Concentration in an industrial evaporator*

Tomato juice was concentrated in a Rossi & Catelli T30 D.F.F. Anteo evaporator (see section 4.2.2.) to solids levels of 10, 15, 20, 25 and 30°Bx. The concentrates were heat processed for 45 min. at 100°C.

*Concentration in a laboratory evaporator*

A Büchi rotary film evaporator was used to concentrate juice samples on laboratory scale. For details see section 4.2.2. Concentrates were heat processed for 15 min. at 100°C.

*Concentration by evaporation under atmospheric conditions*

Juice was concentrated in a beaker at a temperature of approximately 100°C. The juice was stirred continuously during concentration.

*Water removal by gel filtration media*

A dialysis bag (cellulose acetate) filled with tomato juice was dredged with CM-Sephadex (cation-exchanger with sodium as counter-ion, Pharmacia Fine Chemicals, Uppsala, Sweden) and placed in a dessicator at room temperature. The amount of CM-Sephadex was 10% of the juice weight. The concentration process was continued for two days.

*Centrifugation-serum-concentration*

*Laboratory*

6 juice samples of 250 gram were centrifuged for 45 min. at 25,400 x g in a Sorvall RC-5B refrigerated superspeed centrifuge. The clear serum, 6 x 200 gram, was collected and concentrated in a Büchi rotary film evaporator to approximately 30°Bx; the condensate was collected. Equal amounts of serum concentrate were added to the fibres in the centrifuge tubes. To each centrifuge tube different amounts of condensate were added. The fibres, serum concentrate and evaporated water were thoroughly mixed.

*Pilot plant*

Tomato juice aseptically packed in bags was centrifuged at room temperature using a Sharpless P600 Super-D-canter centrifuge at a capacity of 200 kg/hour. The bowl speed of the decanter centrifuge was 6000 rpm

(=3000 x g), the speed difference between screw and drum was 40 rpm, the ring dam was set at the position nearest to the screw shaft. The juice was separated into 25% fibres and 75% serum. The serum was concentrated to 30-35°Bx using a vacuum pan. Fibres and concentrated serum were recombined.

*Factory*

In 1984 a Westfalia CA 220.010 decanter centrifuge was used for the centrifugation of tomato juice at a temperature of 90-92°C and a capacity of 2 tons/hr. The bowl speed of the decanter was 5000 rpm (=3100 x g), the ring dam was set at the position nearest to the screw shaft. The juice was separated in 77% serum and 23% fibre. The serum was concentrated in a stirred vacuum pan (vacuum 66 cm Hg, temperature 52°C, 1900 kg water evaporated/hr.) to 55-60°Bx. The concentrated serum was packed in 5 kg cans at 92°C; the fibres at 94-95°C.

*Concentration of dialysed tomato juice*

Approximately 2 kg of tomato juice was dialysed for 3 days at 4°C against 6 portions of 25 l. distilled water. The dialysed juice was concentrated using a Büchi laboratory rotary film evaporator either with or without the addition of the previously dialysed sugars. The same tomato juice was also concentrated without prior dialysis.

*Concentration of PE treated tomato juice*

1.5 kg of tomato juice was incubated for 24 hours at 37°C with 64 units of pure mould PE. PE treated as well as untreated juice was concentrated using a Büchi laboratory rotary film evaporator.

## 7.3. *Results and Discussion*

### 7.3.1. *Effect of hot break temperature on gross viscosity*

The influence of the hot break temperature on the gross viscosity of tomato juice and its concentrates was studied using two types of scalders: 1) a conventional Rossi & Catelli 1000 L scalder and 2) a modern Rossi & Catelli "Eldorado" scalder.

Table 58: Influence of hot break temperature on viscosity and composition of tomato juice (1983).

| HB temp. | °Bx | % TS | % WIS | Bostwick | $\eta_{app}$ [1] | | $\eta_{serum}$ | WIS composition (% w/w) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | conc. | dil. | | AGA | DE AGA | protein | cellulose |
| 90 | 4.7 | 5.44 | 0.71 | 20.5 | 92.9 | - | 1.5 | 10.9 | 13 | 20.3 | 35.4 |
| | - | - | 2.00 | 3.5 | 762.6 | - | - | | | | |
| | 17.4 | 19.92 | 2.69 | <1 | 1457.0 | 63.7 | 4.9 | | | | |
| 97 | 5.3 | 5.83 | 0.86 | 14.6 | 145.8 | - | 1.8 | 11.4 | 22 | 20.1 | 36.6 |
| | - | - | 2.00 | 3.6 | 794.7 | - | - | | | | |
| | 16.9 | 20.08 | 2.81 | <1 | 1552.6 | 103.8 | 9.0 | | | | |
| 103 | 5.4 | 6.10 | 1.02 | 12.3 | 187.7 | - | 2.6 | 12.2 | 35 | 19.7 | 35.0 |
| | - | - | 2.00 | 3.8 | 760.2 | - | - | | | | |
| | 17.5 | 18.62 | 3.02 | <1 | 1753.2 | 133.5 | 21.7 | | | | |

1: conc. = gross viscosity of concentrate; dil. = gross viscosity of concentrate diluted to juice strength.

Table 58 and Figures 45 and 46 present the results of experiments carried out for two succesive years (1983, 1984) with the R&C 1000 L scalder. For both years the gross viscosity increased significantly upon an increase in hot break temperature from 97-98°C to 103-105°C. In 1983 the increase in hot break temperature from 90°C to 97°C was also found to affect the gross viscosity.

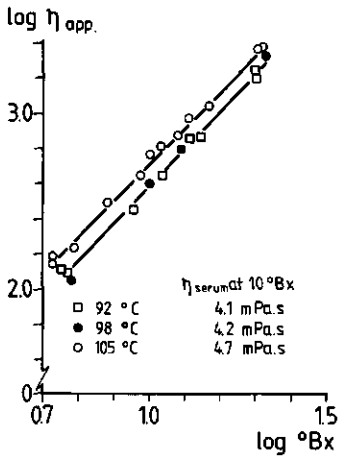



Fig. 45: Effect of hot break temperature on gross viscosity and serum viscosity in relation to °Bx (1984).

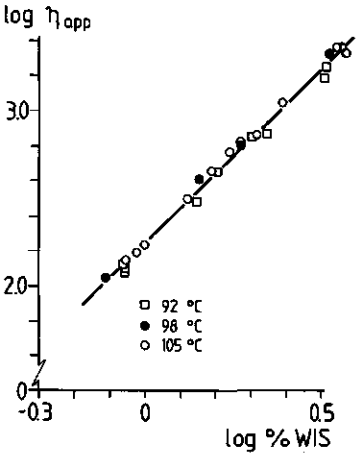



Fig. 46: Effect of hot break temperature on gross viscosity in relation to the WIS content (1984).

This increase in gross viscosity as a result of an increase in hot break temperature has often been attributed to quicker enzyme inactivation (Luh et al. 1981, 1982, 1984a,b,c) and better removal of insoluble solids from skin and seed material (Whittenberger & Nutting 1957, Twigg 1959). Indeed, the data shown in Table 58 on the serum viscosity of the samples and the DE of the WIS pectin seem to indicate that a quicker enzyme inactivation is involved in the increase in gross viscosity. Considering the data presented in chapter 4 (comparison of hot break (PHB) with cold break (PCB)) and chapter 6 (action of PE + PG on tomato concentrate), it is hard to believe that the tomato enzymes were able to degrade 20% of the WIS in the relatively short time between crushing and heating to 90°C.
Microscopic examination of the juices did not show any changes in the

appearence of the cell walls as a result of the hot break at different temperatures; this is in contrast to results presented by Xu et al. (1986). Moreover results presented in table 58 show that the quality of the WIS is not affected by the hot break temperature; all juices showed comparable gross viscosity at 2% WIS. Apparently the enzymes degraded the serum pectin and partly saponified the WIS pectin, however, no indications were found for WIS degradation at the lower hot break temperature.
In 1984 it was found that the serum viscosity increased only slightly as a result of the increases in hot break temperature; this indicates that the pectolytic enzymes were effectively inactivated even at a hot break temperature of 92°C. (Fig. 45). Therefore the higher gross viscosity found as a result of an increase in hot break temperature from 92-98°C to 105°C is most likely caused by increased tissue softening and better removal of WIS from skin and seed material. Again the quality of WIS was not affected by hot break temperature. (Fig. 46).

In the hot section tank of the R&C "Eldorado" scalder the ratio between recirculation rate of heated tomatoes and feed rate of fresh tomatoes is approximately 20. Fresh tomatoes can be heated almost instantaneously to temperatures high enough for enzyme inactivation. In the experiments this temperature was measured at the inlet of the tomatoes in the hot section tank. The final product temperature was measured at the point where the recirculated heated tomatoes entered the hot section tank.

Table 59: Effect of hot break temperature on viscosity and WIS content of tomato paste (1984).

| temperature hot section tank | | analysis of paste diluted to 12.5°Bx | | | |
|---|---|---|---|---|---|
| inlet | after tub. heater | % WIS | $\eta_{app}$ | $\eta_{serum}$ | Bostwick |
| 87 | 91 | 2.70 | 969 | 6.9 | 2.8 |
| 95 | 101 | 2.46 | 815 | 6.5 | 2.0 |
| 98 | 107 | 3.09 | 1443 | 7.6 | 1.2 |
| 104 | 109 | 2.81 | 1181 | 7.8 | 1.3 |

Tables 59 and 60 show the results of the experiments with this type of scalder. The Bostwick values were determined directly in the factory on paste sampled at the outlet of the R&C T60 Califfo evaporator. °Bx, %TS, WIS, $\eta_{app}$ and $\eta_{serum}$ were determined in the laboratory on aseptically filled paste.

Table 60: Effect of hot break temperature on solids content and gross viscosity of tomato paste (1985).

| temperature hot section tank | | solids content paste | | | Bostwick diluted paste* | | $\eta_{serum}$ |
|---|---|---|---|---|---|---|---|
| inlet | after tub. heater | °Bx | %TS | %WIS | 12.5°Bx | 8.3°Bx | 10°Bx |
| 90 | 92 | 23.5 | 24.78 | 4.30 | 2.7 ± 0.3 | 7.2 ± 0.5 | 9.0 |
| 94 | 98 | 23.4 | 24.87 | 4.52 | 2.3 ± 0.3 | 6.5 ± 0.6 | |
| 100 | 105 | 23.5 | 24.85 | 4.30 | 2.1 ± 0.3 | 6.2 ± 0.5 | |
| 105 | 108 | 23.6 | 25.42 | 4.85 | 1.7 ± 0.3 | 5.8 ± 0.4 | |
| 109 | 115 | 23.0 | 24.73 | 4.70 | 1.6 ± 0.1 | 5.4 ± 0.2 | 10.6 |

* data are a mean value of 8-10 measurements.

With the "Eldorado" scalder no large differences in gross viscosity were found between the use of hot break temperatures of 91-92°C and 101-105°C (final temperatures), which is in contrast to results obtained with the R&C 1000 L scalder. Hot break temperatures in excess of 105°C, however, did result in increased WIS content and gross viscosity. It has to be noted that in Italy firm, high viscosity varieties are used for processing. Apparently it is more difficult to remove the WIS from the skin material of these varieties than from the skin material of the medium viscosity varieties used in Portugal. Microscopic examination did not indicate cell wall breakdown at low or high hot break temperatures, WIS quality was not affected by the degree of the hot break temperature. Moreover, data on serum viscosity showed that pectolytic enzymes were effectively inactivated. The results obtained with the R&C "Eldorado" scalder also point to the improved removal of WIS from skin and seed material as a cause of increased gross viscosity at high hot break temperatures.

### 7.3.2. *Effect of finishing on gross viscosity*

Crushed tomatoes of the variety H30 were prepared at hot break temperatures of 90°C and 103°C. Part of the crushed tomatoes was finished with an industrial R&C paddle type finisher at approximately the hot break temperature, another part was finished using a laboratory Bauknecht KU 2-1 tapered screw juice extractor at 90°C.
Fig. 47 shows that higher gross viscosities of juice and concentrates were obtained when crushed tomatoes were finished with the industrial paddle finisher. This difference can be attributed to a 23% (hot break 103°C) and 36% (hot break 90°C) higher WIS content in the juice finished with the paddle finisher. (Table 61).

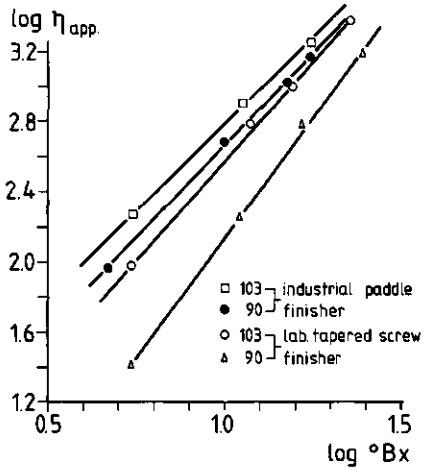



Fig. 47: Effect of type of finisher on gross viscosity in relation to °Bx.

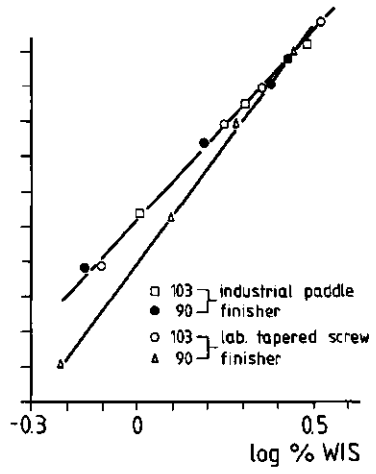



Fig. 48: Effect of type of finisher on gross viscosity in relation to WIS content.

Similar results were found when non heat processed samples, prepared at a hot break temperature of 103°C, were used in these experiments.
Obviously the paddle finisher is much more efficient in removing WIS from the skin material. The chemical composition of the WIS in juices finished with the tapered screw finisher was not significantly different from the chemical compositions shown in Table 58 for juices finished with the paddle finisher. The increase of the WIS content at higher hot break temperatures

(34% for tapered screw extractor, 20% for paddle finisher) cannot be attributed to an increased finishing temperature. Cooling the tomatoes, crushed at 103°C, to a temperature of 90°C or 65°C before finishing with the paddle finisher did not result in decreased gross viscosity. Moreover, differences in juice gross viscosity were also found after finishing tomatoes, crushed at either 90°C or 103°C, with the screw type finisher at a temperature of 90°C.

Table 61: Viscosity and solids content of juices (at 5°Bx) finished with two types of finisher.

| hot break temp. | finisher | %TS | %WIS | $\eta_{app}$ | $\eta_{serum}$ | Bostwick |
|---|---|---|---|---|---|---|
| 90°C | tapered screw | 5.40 | 0.56 | 20.2 | 1.4 | >24 |
| 90°C | paddle | 5.70 | 0.77 | 106.7 | 1.6 | 18.7 |
| 103°C | tapered screw | 5.74 | 0.75 | 78.5 | 2.4 | 20.4 |
| 103°C | paddle | 5.78 | 0.92 | 157.3 | 2.4 | 13.3 |

Preheat treatment exerts an important influence on the finisher operation, especially when a less efficient finisher (tapered screw) is used. When tomatoes, crushed at 90°C, were finished with this tapered screw juice extractor the juice not only contained less WIS (Table 61) but also WIS of an inferior quality (Fig. 48). It is interesting to note that the heat processing at 100°C of tomatoes crushed at 90°C, does not result in an improved removal of WIS from skin material during finishing. Apparently, a combination of the higher temperature and mechanical shear (recirculation in the chop/scalder) is needed for the loosening of WIS from skin material.

Table 62: Viscosity and solids content of juice (at 5°Bx) finished at 20°C using the tapered screw finisher.

| hot break temp. | %TS | %WIS | $\eta_{app}$ | $\eta_{serum}$ | Bostwick |
|---|---|---|---|---|---|
| 90°C | 5.40 | 0.68 | 37.2 | 1.3 | >24 |
| 103°C | 5.66 | 0.80 | 99.6 | 2.2 | 17.5 |

Surprisingly, the efficiency of the tapered screw finisher increased when the crushed tomatoes were finished at 20°C instead of 90°C. (Tables 61, 62). The quality of the WIS in juice prepared at a hot break temperature of 90°C did not increase however.

Fresh $H_{30}$ tomatoes were heated in closed sterilization bags in a retort to a heart temperature of 90°C. The hot tomatoes were finished using the tapered screw finisher equipped with a 0.5 mm or 1.5 mm screen. The total yield increased from 93% to 96% when the 1.5 mm screen was used. At a screen size of 1.5 mm a 22% increase in WIS content was found (Table 63), resulting in increased gross viscosity (Fig. 49).

Table 63: Viscosity and composition of juices (at 5°Bx) finished using different screen openings.

| | finisher screen size | |
|---|---|---|
| | 0.5 mm | 1.5 mm |
| $\eta_{app}$ | 78.7 | 97.2 |
| $\eta_{serum}$ | 2.3 | 2.3 |
| Bostwick | 21.2 | 19.1 |
| %TS | 5.71 | 5.64 |
| %WIS | 0.73 | 0.89 |
| AGA[1] | 15.7 | 16.2 |
| DE | 32 | 35 |
| Cellulose[1] | 33.8 | 31.0 |
| Protein[1] | 17.1 | 17.0 |

1: expressed as % of WIS

In spite of this increase, the quality of WIS obtained using a 1.5 mm screen size was not as good as the quality of the WIS obtained using a 0.5 mm screen: this was seen predominantly at a higher WIS content (Fig. 50). The chemical composition of the WIS was not affected by screen size. (Table 63).

Using the more efficient three stage paddle finisher, no effects of

finisher screen size (0.8-1.0-1.5 mm final screens) on the WIS content and gross viscosity of tomato paste was found (Fig. 51).

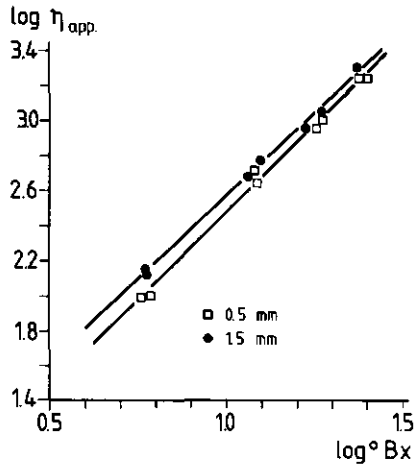



Fig. 49: Effect of finisher screen opening on the gross viscosity in relation to °Bx (tapered screw finisher).

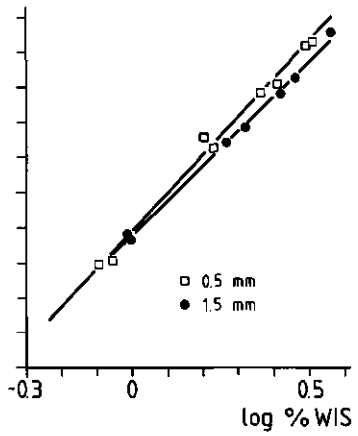



Fig. 50: Effect of finisher screen opening on the gross viscosity in relation to WIS content (tapered screw finisher).

The effects of preheat treatment, finisher temperature and finisher screen opening on the finisher operation were shown to depend on the type of finisher. In confirmation with results presented by other authors (Hand et al. 1955, Bel-Haj 1981) it was found that a paddle type finisher is more efficient in removing WIS from skin material than a screw type finisher. The positive effect of a higher preheating temperature on the gross viscosity and WIS content of juice was, therefore, much more pronounced for the less efficient screw type finisher.

Hand et al. (1955) reported a decrease in juice gross viscosity as a result of a decrease in finishing temperature after a hot break at 93°C. Our results show that, with an efficient paddle finisher, a decrease in finishing temperature does not influence the WIS content and gross viscosity of tomato paste. With the less efficient screw type finisher, a finishing temperature of 20°C resulted in higher WIS content and gross viscosity of juice when compared to finishing at 90°C. The stiffness of the particles at 20°C obviously influenced the removal of WIS from skin material.

The gross viscosity and WIS content of paste was not influenced by the size of the finisher screen openings (range 0.8-1.5 mm) when crushed tomatoes were finished using the paddle finisher. The size of the finisher screen openings did, however, influence the gross viscosity and WIS content of juice when the tapered screw finisher was used.

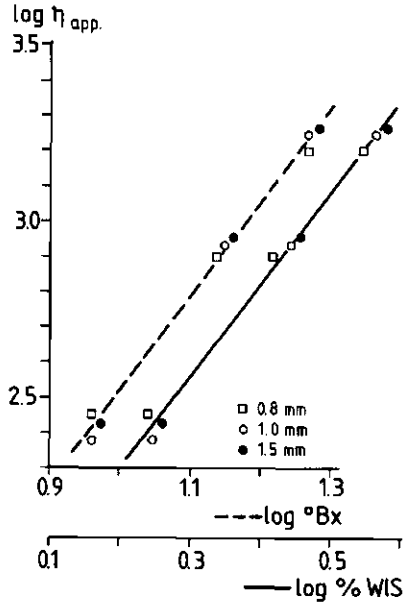



Fig. 51: Effect of finisher screen opening on the gross viscosity (paddle finisher) of dilutions of tomato paste.

In general, the data in the literature point to an increase in the gross viscosity of juice, paste and ketchup as result of an increase in size of the finisher screen opening (Hand et al. 1955, Smit & Nortje 1958, Moyer et al. 1959, Twigg 1959, Bel-Haj 1981).

### *7.3.3. Effect of concentration on gross viscosity*

In order to establish the potential effect of the concentration process on tomato paste gross viscosity, tomato juice of the variety H30 was concentrated using different methods or different types of equipment. Figure 52 shows that the method of concentration or the type of equipment

had no influence on the gross viscosity of the concentrates. From the data in Fig. 23 it was already concluded that "WIS-concentration" (section 4.2.2.) resulted in the same gross viscosity of concentrates as concentration with the laboratory rotary film evaporator. Obviously the recovery of gross viscosity is not improved by using a relatively mild laboratory concentration technique instead of an industrial evaporator. In spite of some solubilization of WIS pectin, in general the composition of WIS and the WIS/TS ratio were found to remain constant during concentration. The solubilization of pectin seems to depend on the degree of esterification of the WIS pectin: juice samples of the varieties 722 and 318 with a DE of 52% lost up to 25% of the AGA content (equal to 4% of the WIS) upon concentration with a Pfaudler wiped film evaporator. On the contrary, juice samples of the variety H30 with a DE of the WIS pectin of 29 lost almost no AGA. As reported above (Table 36) the hemicellulose present in WIS did not change during concentration.

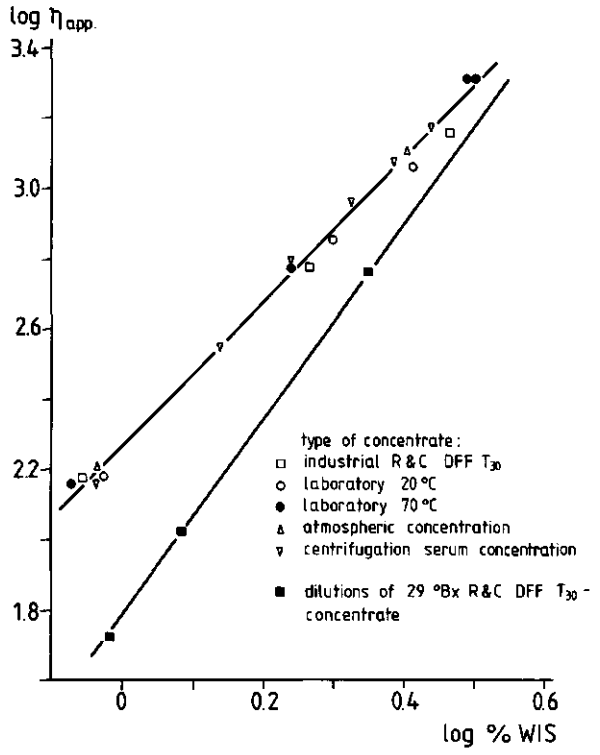



Fig. 52: Gross viscosity of juices and concentrates prepared according to different methods; gross viscosity of dilutions of an industrial concentrate.

Figure 52 also shows a significant difference between the gross viscosity of juice and concentrates on the one hand and the gross viscosity of diluted paste at the same solids level on the other hand. This difference in gross viscosity will be called the "dilution-loss". There were no indications that concentration affects the serum viscosity of diluted concentrates.

As can be seen in Fig. 52, a "dilution-loss" of 65-70% was found when tomato juice of the variety H30 was concentrated to 29°Bx using the R&C $T_{30}$ Anteo evaporator and then diluted to the original strength. Comparable "dilution-losses" were found after the dilution of tomato paste samples concentrated in the R&C T60 Califfo evaporator (samples decribed in section 7.3.1.). The dilution of 23°Bx paste samples concentrated using the laboratory rotary film evaporator ($\eta_{app}$ of concentrates is shown in Fig. 52) resulted in dilution losses of only approximately 30% irrespective of the temperature of concentration (20°C or 70°C). Even under atmospheric conditions during evaporation the "dilution-loss" did not increase to a level higher than 35%.

The effect of the centrifugation-serum-concentration process on the "dilution-loss" will be discussed later. The results indicate that the magnitude of the "dilution-loss" strongly depends on the method of concentration; the temperature of evaporation seems to have no influence. The "dilution-loss" is also influenced by the solids content of the concentrate.

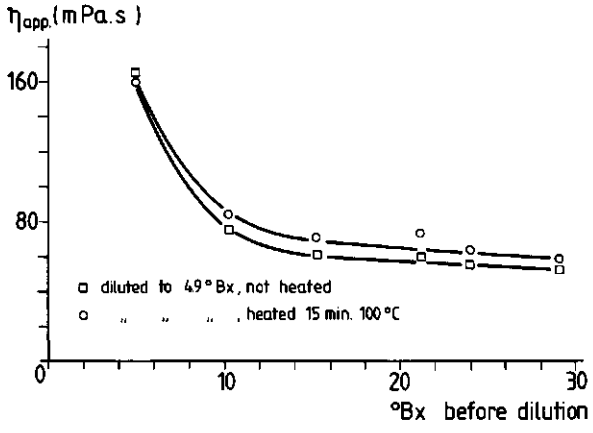



Fig. 53: Gross viscosity after dilution of concentrates to 4.9°Bx; effect of additional heating of dilutions on gross viscosity.

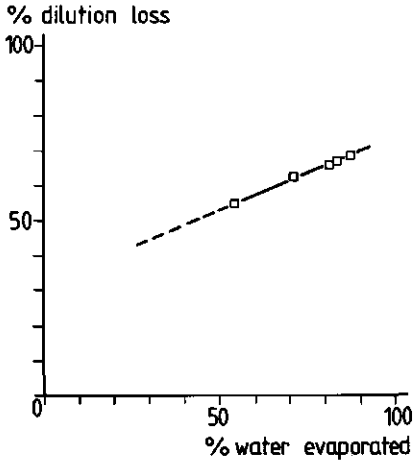



Fig. 54: % "dilution-loss" as function of the % water evaporated during concentration.

Figures 53 and 54 show the "dilution-loss" of tomato juice concentrated to various solids levels using the R&C T30 Anteo evaporator ( $_{app}$ of concentrates is shown in Fig. 52). Using this evaporator the "dilution-loss" was found to be proportional to the amount of water evaporated (Fig. 54). Additional heating of the diluted samples for 15 min. in boiling water did not negate the "dilution-loss"; it resulted in only a small gross viscosity increase (Fig. 53). The same was found when dilutions of laboratory concentrated samples were heated.

The degree of sedimentation (section 3.4.) of WIS particles in relation to WIS concentration for unconcentrated tomato juice used in this study is shown in Fig. 55. The juice was diluted with different amounts of water and transferred to 100 ml graduated serum bottles. After 7 days the volume of the sediment was recorded. The WIS particles filled the complete volume at a concentration of ± 0.65 g/100 ml. Samples of the juice concentrated to several solids levels with the R&C T30 Anteo evaporator (Fig. 53 and 54) were diluted to 4.9°Bx (0.95% WIS), which is the refractive index of the juice before concentration.

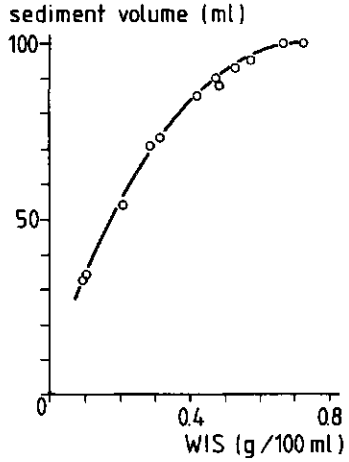



Fig. 55: Degree of sedimentation of WIS particles in relation to WIS concentration.

50 grams of each dilution was weighed into the serum bottles and adjusted with water to a final volume of 100 ml. The sedimentation volume measured after 7 days clearly decreased as the concentration of the paste before dilution increased (Fig. 56).

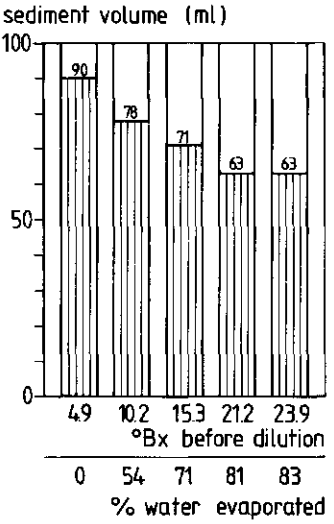



Fig. 56: Sedimentation volume of WIS particles (0.475 g/100 ml) in relation to concentration of tomato juice.

Comparison of Fig. 55 and Fig. 56 shows that the sedimentation volume obtained with 0.475 g WIS/100 ml as present in the 23.9°Bx concentrate, equals the sedimentation volume of only 0.24 g WIS/100 ml as present in unconcentrated juice. Microscopic examination of the dilution of the 23.9°Bx concentrate showed a considerable amount of collapsed and crumpled cells (Fig. 57).

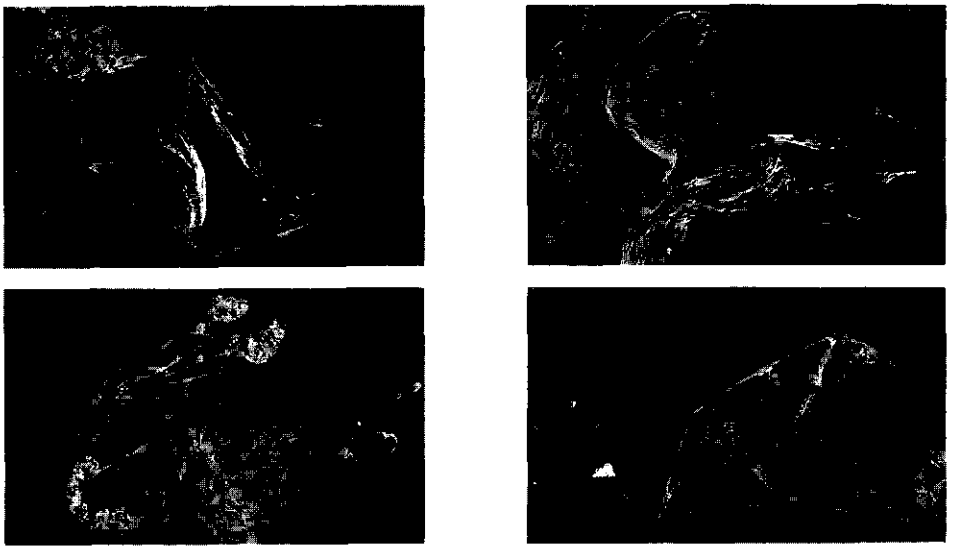


Fig. 57: Collapsed and crumpled cells as present in diluted industrial tomato paste (magnification 61.4x).

Apparently those cells were not able to reabsorb the water which was evaporated initially. This effect could also be demonstrated by the use of the density gradient centrifugation technique (section 3.4.).

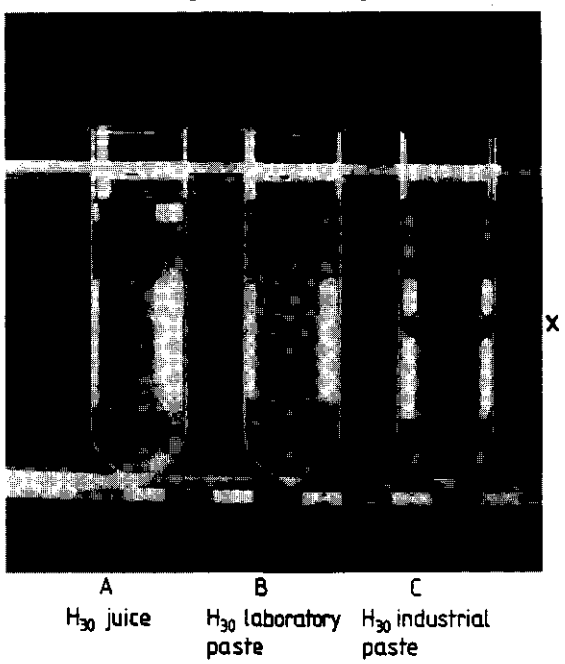



Fig. 58: Density gradient centrifugation of tomato juice and paste samples.

Figure 58 shows typical centrifugation patterns for: hot break juice (A), a diluted laboratory concentrate of A (B) and a diluted industrial concentrate of A (C). As a result of the concentration and dilution steps, a band X appears in sample C which is not present in sample A. This band consists almost entirely of collapsed and crumpled cells. Band X was found to be barely present in sample B. The "dilution-loss" of this sample (± 30%), however, was rather small when compared to the 60% "dilution-loss" of sample C.

It is obvious that there is a positive correlation between the magnitude of the "dilution-loss" and the presence of collapsed and crumpled cells. This also explains why concentration curves ($\eta_{app}$ vs. %WIS) are not dependent on method of concentration. During concentration, tomato cells collapse and crumple irrespective of the method of concentration. In this situation gross viscosity is determined only by the amount of WIS.

It is not known by which mechanism the collapsed cells have lost the ability to reabsorb water and to expand. The influence of vacuum, shear and water removal on the determined "dilution-loss" was studied using the rotary vacuum film evaporator (Table 64). Juice of the variety H1361 was concentrated at a product temperature of 20°C and at two levels of shear. Moreover the juice was treated in the evaporator under such conditions that no evaporation occurred. In this way the influence of shear, vacuum and the combination of both factors on juice gross viscosity was studied. The gross viscosity of the juices and diluted paste samples was measured two days after the treatment.

Table 64: Influence of different treatments in a laboratory rotary film evaporator on gross viscosity of tomato juice and diluted tomato paste.

| sample | product temp. | vacuum | evaporation | rotation (rpm) | % dilution/ viscosity loss |
|---|---|---|---|---|---|
| 1 | 70 | + | + | 63 | 42 |
| 2 | 70 | + | + | 158 | 51 |
| 3 | 65 | + | - | 63 | 21 |
| 4 | 65 | + | - | 158 | 22 |
| 5 | 65 | - | - | 63 | 17 |
| 6 | 65 | - | - | 158 | 19 |
| 7 | 65 | + | - | - | 12 |

From the data it is evident that the loss in gross viscosity due to evaporation, measured as "dilution-loss", is caused by a complex of factors such as water-removal, mechanical shear and vacuum. Results shown in Table 64 also indicate that a part of the "dilution-loss" is not caused by evaporation but solely by vacuum and mechanical shear. In Fig. 59 the gross viscosity of dilutions of laboratory concentrates is plotted against the WIS content before dilution. Apparently the real "dilution-loss" starts at 1.5-1.6% WIS; at this point approximately 40% of the water was evaporated. A loss in gross viscosity at WIS levels lower than 1.5-1.6% was also found

when tomato juice was stirred in the rotary film evaporator without being concentrated. These results were confirmed by repeating the experiment using a different tomato juice. Figure 59 also reveals that concentrates of juice from which ions and low molecular weight sugars were removed by dialysis showed "dilution-losses" comparable to the viscosity loss induced by vacuum and mechanical shear. (see also Tables 26 to 28). Replenishment of the sugars removed by dialysis prior to concentration did not result in an increase of the "dilution-loss". This result indicates that the ions removed during dialysis are potentially important with regard to "dilution-loss". It was found that approximately 50% of the calcium present in juice could be removed by dialysis.

The treatment of tomato juice with purified PE (no PG or PL activity present) resulted in an increase in the gross viscosity of the juice and in gel formation upon storage.

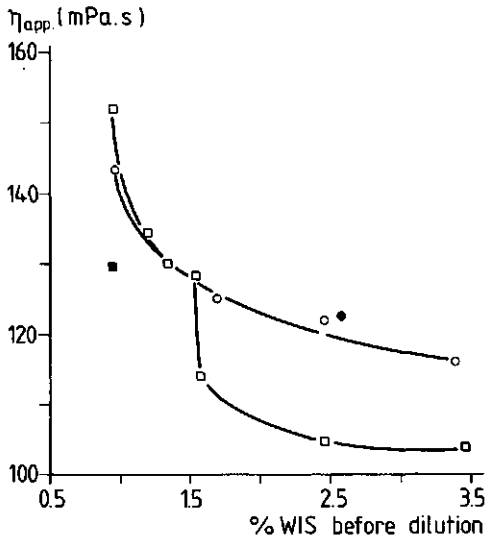



Fig. 59: Gross viscosity after dilution of concentrates to 0.95% WIS. Effect of stirring in evaporator on gross viscosity.
- □ dilutions laboratory concentrate
- ■ juice stirred in evaporator without concentration
- ○ dilutions of laboratory concentrates of dialysed juice
- ● dilution of laboratory concentrate of dialysed juice + removed sugars

Concentrates of PE treated juice were also higher in gross viscosity than concentrates of untreated juice (Table 65).

Table 65: Influence of treatment with PE on viscosity and solids content of tomato juice and concentrates.

| | °Bx | %TS | %WIS | $\eta_{app}$ | $\eta_{serum}$ |
|---|---|---|---|---|---|
| Blank | 5.3 | 6.16 | 0.97 | 169 | 2.4 |
| | 9.9 | 11.26 | 1.74 | 569 | 5.1 |
| | 16.4 | 18.32 | 2.85 | 1543 | 15.6 |
| PE | 5.3 | 6.23 | 0.97 | 202 | 1.2 |
| | 9.8 | 11.35 | 1.74 | 664 | 1.9 |
| | 17.2 | 19.48 | 3.12 | 2771 | 2.6 |

Dilution of the most concentrated samples resulted in a "dilution-loss" of 25% in the untreated sample and 75% in the PE treated sample. Microscopic examination showed a large amount of collapsed cells in the dilution of the PE treated sample. The sedimentation volume of this sample was 51 ml at 0.475 g WIS/100 ml compared to 81 ml for the untreated sample (see also Fig. 55). Obviously the increase in calcium reactive carboxyl groups caused by PE action resulted in a very large "dilution-loss". It is interesting to note that serum viscosity decreased as result of treatment with PE. Apparently a large part of the saponified serum pectin precipitated in the WIS. The precipitation of saponified serum pectin has already been shown in section 4.3. (samples LHB, $LCB_1$).

In the course of these studies an attempt was made to concentrate tomato juice without mechanical shear and vacuum by water removal with ionic and non-ionic gel filtration media. (Table 66). 1.5 gram of Sephadex was dredged on a dialysis bag filled with 10 grams of juice. The decrease in weight of the juice and dialysis bag was determined after 2 days. The weak cation exchanger CM-Sephadex was found to have the highest "concentration capacity". In a second experiment 600 grams of juice was concentrated, again by dredging CM-Sephadex (sodium as counter-ion) on a dialysis bag filled with the juice.

Table 66: Concentration of tomato juice with ionic and non-ionic gel filtration media (Sephadex, Pharmacia Fine Chemicals, Uppsala, Sweden).

| | degree of concentration |
|---|---|
| Sephadex G50 | 1.5 |
| Sephadex G100 | 5.3 |
| Sephadex G150 | 3.6 |
| Sephadex G200 | 4.5 |
| Sephadex-DEAE | 2.0 |
| Sephadex-QAE | 2.1 |
| Sephadex-CM | 14.6 |
| Sephadex-SP | 4.7 |

After two days the juice was concentrated 11.1 times; this means that this paste was comparable to a 60°Bx hot break paste.

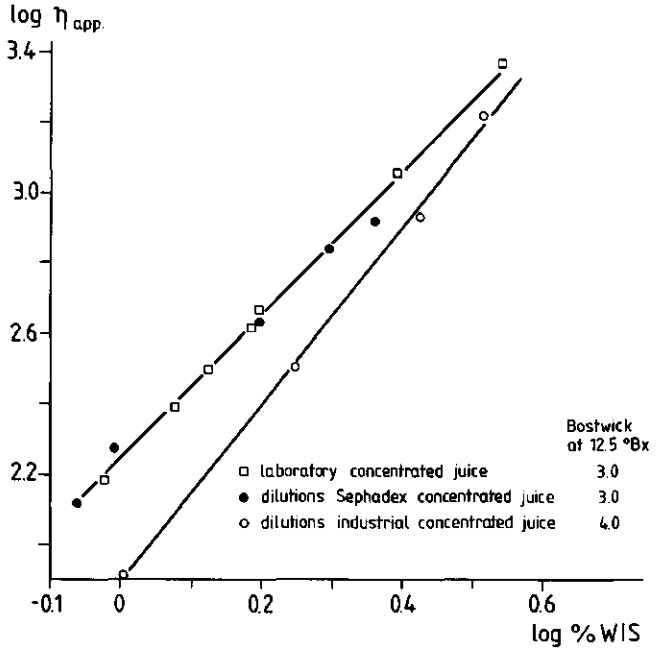



Fig. 60: Gross viscosity of dilutions of industrially concentrated juice and Sephadex concentrated juice compared to gross viscosity of juice concentrates.

Upon the dilution of samples concentrated according to this technique, no "dilution-loss" was observed (Fig. 60). Moreover, microscopic examination showed no significant difference in amount of collapsed cells between untreated juice and dilutions of Sephadex concentrated juice. This observation leads to the conclusion that water removal as such does not cause a "dilution-loss". Another indication for this conclusion was found after the centrifugation of tomato juice in a Sorvall RC-5B refrigerated superspeed centrifuge for 45 min. at 25,400 x g. Resuspension of the serum and fibres (respectively 75% and 25% of total weight) resulted in only small gross viscosity losses of between 0% and 10%.
Table 67 shows the results of a laboratory centrifugation-serum-concentration experiment. No "dilution-losses" were found. The gross viscosity of these samples before dilution was the same as the gross viscosity of other types of concentrates (Fig. 52).

Table 67: Gross viscosity of concentrates, prepared according to the centrifugation-serum-concentration technique, before and after dilution*.

| concentrates | | | dilutions of 4.9 °Bx |
|---|---|---|---|
| °Bx | %WIS | $\eta_{app}$ | $\eta_{app}$ |
| 4.9 | 0.92 | 145.0 | 152.6 |
| 7.1 | 1.38 | 355.5 | 148.0 |
| 9.5 | 1.74 | 627.4 | 148.0 |
| 11.6 | 2.12 | 912.1 | 148.5 |
| 13.4 | 2.44 | 1192.8 | 146.7 |
| 15.0 | 2.74 | 1490.8 | 151.2 |

* dilutions were made 1 day after preparation of the concentrates.

In summary it can be concluded that "dilution-losses" are observed when certain serum components (ions) are concentrated by evaporation in the presence of tomato cells. The magnitude of the "dilution-loss" is further influenced by mechanical shear and vacuum.

The results obtained indicated that the centrifugation-serum-concentration technique could be a solution for the prevention of "dilution-loss". Two types of decanter centrifuges were used to test the usefulness of this technique on a larger scale. The results obtained with the different types of decanter were comparable; a typical result is shown in Fig. 61.

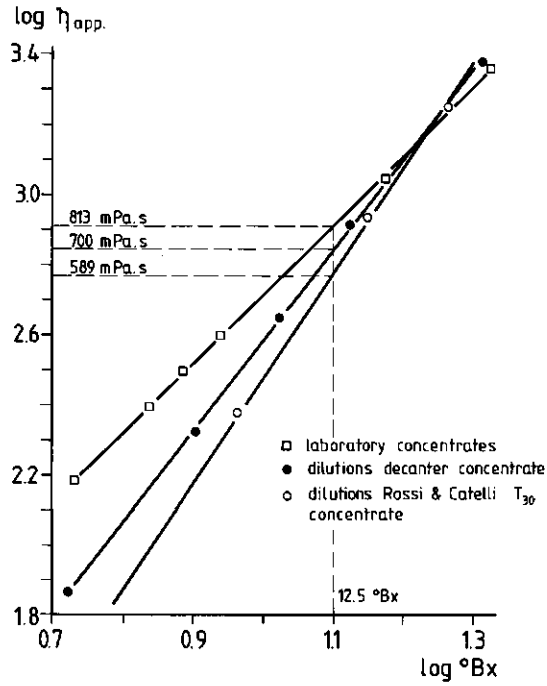



Fig. 61: Gross viscosity of dilutions of industrially concentrated juice and juice concentrated according to the centrifugation - serum - concentration technique compared to gross viscosity of juice concentrates.

Dilutions of the concentrate prepared in the R&C T30 Anteo evaporator show a "dilution-loss" of 28% when diluted to a soluble solids level of 12.5%. At this solids level a concentrate prepared using the decanter showed a "dilution-loss" of 14%. Bostwick values at 12.5% soluble solids for evaporator paste and decanter paste were respectively 5.5 cm and 4.4 cm. Under specified conditions the use of a decanter in the centrifugation-serum-concentration process resulted in a significantly

reduced "dilution-loss", although, a complete prevention of the "dilution-loss" could not be achieved. Obviously the centrifugation of tomato juice in a decanter cannot be directly compared to the centrifugation of tomato juice in a bench top centrifuge. An explanation for the difference between the two centrifuges can be found in the higher mechanical shear on the insoluble particles during centrifuging in a decanter. The results presented in this study on the effect of the centrifugation-serum-concentration technique on the gross viscosity of tomato paste are contradictory to results published by other authors (Ephraim et al. 1962, Kopelmann & Mannheim 1964, Mannheim & Kopelmann 1964, Harper & El Sahrigi 1965). Harper & El Sahrigi were the only authors who used a hot break juice for the centrifugation but unfortunately they did not describe the centrifugation process in detail.

The "dilution-loss" is influenced not only by the concentration process but also by other processing factors, as will be shown in section 7.3.4. and in the following example. Whole tomatoes were heated in closed sterilization bags in a retort for 20 min. at 120°C (control). A heart temperature of 90°C was measured.

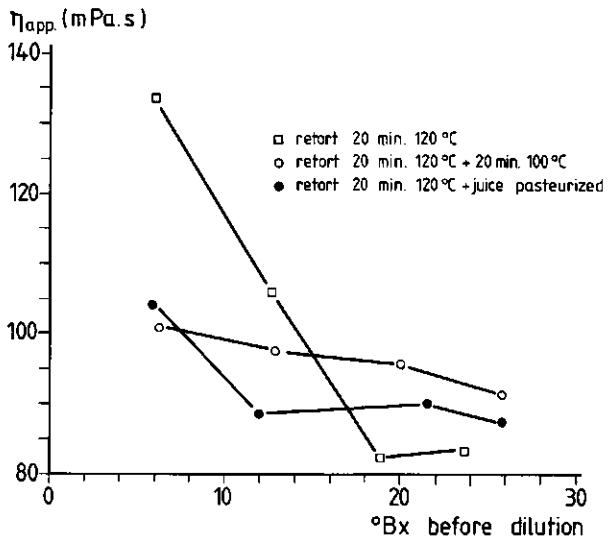



Fig. 62: Effect of additional heat treatment on gross viscosity of juice and diluted concentrates.

The heated tomatoes were finished through a Bauknecht KU 2-1 tapered screw juice extractor equipped with a 1.5 mm screen. The chemical composition of this juice was shown in Table 63. Half of the finished juice was heat processed for 100 min. at 100°C. In another experiment, tomatoes were heated for 20 min. at 120°C and then the retort temperature was reduced to 100°C; heating was continued for 20 min. The heated tomatoes were finished in the same way as described above.

Both additional heat treatments, on top of the 20 min. at 120°C, gave rise to a reduction in gross viscosity of ± 25% (Fig. 62), caused by the solubilization of 20-25% of the AGA present in the WIS. Compared to the "dilution-loss" for the control, these additional heat treatments caused only small "dilution-losses"; this was due to the very low gross viscosity of the juice before evaporation.

There were no indications that the degree of hot break temperature influences the magnitude of the "dilution loss". Tomato paste samples prepared at different hot break temperatures (Table 59) showed, after dilution, identical gross viscosity at a specific WIS content, irrespective of the hot break temperature (Fig. 63).

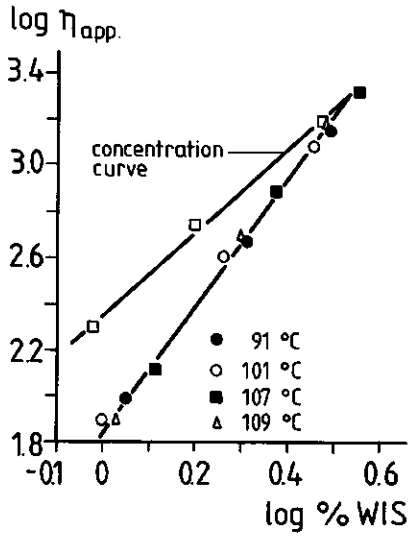



Fig. 63: Gross viscosity of dilutions of 23°Bx tomato paste prepared at different hot break temperatures compared to gross viscosity of concentrates.

### 7.3.4. *Effect of heat processing and freezing on gross viscosity*

Table 68 shows the effect of pasteurization on the gross viscosity of non-pasteurized hot break tomato juice and concentates.
A 10% reduction in gross viscosity of a 15.8°Bx tomato paste was observed due to heat processing for 100 min. at 100°C. Upon dilution of this sample a "dilution-loss" of 48% was found; in the case of non-pasteurized paste, a "dilution-loss" of only 29% was found.

Table 68: Effect of pasteurization of tomato juice and concentrates on gross viscosity before and after dilution*.

| | before pasteurization | | | after pasteurization | | |
|---|---|---|---|---|---|---|
| | °Bx | %WIS | $\eta_{app}$ | °Bx | %WIS | $\eta_{app}$ |
| concentrates | 4.8 | 0.95 | 143.1 | 4.8 | 0.91 | 124.8 |
| | 9.4 | 1.79 | 573.2 | 9.4 | 1.78 | 530.2 |
| | 15.8 | 3.00 | 1609.9 | 15.8 | 2.89 | 1471.3 |
| | 18.7 | 3.49 | 2211.8 | | | |
| dilutions | 9.4 → 4.8 | 0.90 | 102.8 | 4.7 | 0.91 | 82.0 |
| | 15.8 → 4.8 | 0.92 | 101.4 | 4.7 | 0.91 | 74.3 |
| | 18.7 → 4.8 | 0.88 | 99.0 | | | |

* Concentrates were obtained in a rotary film evaporator on laboratory scale; an aliquot of the concentrates was heat processed (100 min. at 100°C); measurements were done either directly on diluted non-pasteurized samples or on pasteurized samples which were diluted. Juice of the variety H30 was used.

Similar effects of pasteurization on the gross viscosity of tomato paste were observed when hot break paste was produced in Italy for the purpose of the experiments described in section 7.2.2. Tomato paste was sampled at the outlet of the Rossi & Catelli T60 Califfo evaporator (non-sterilized paste) and at the aseptic filler (paste sterilized for 2 min. 15 sec. at 109°C). Comparison of gross viscosity data for non-sterilized and sterilized paste

indicated that Bostwick values at 12.5°Bx were 0.5-1.0 cm. higher for the sterilized paste. Heat processing seems to increase the "dilution-loss" of a tomato concentrate. These results confirm the results reported by Marsh et al. (1977, 1982).

The loss in gross viscosity of a juice upon pasteurization (Fig. 62, Table 68) can be related to a decrease in WIS content, caused mainly by the solubilization of WIS pectin. This quantity of solubilized WIS seems to depend on the amount of AGA present in the WIS relative to the other constituents (e.g. AGA/Cellulose ratio). Industrially prepared samples of juice, in general, show a AGA/Cellulose ratio of 0.30-0.35 and will lose approximately 4-5% of the WIS as a result of heat processing. The laboratory prepared juice sample shown in Fig. 62 had a relatively high AGA/Cellulose ratio of 0.52 due to the effective "in-situ" inactivation of enzymes in the retort. This sample lost almost 10% of the WIS and showed a 25% decrease in gross viscosity as a result of heat processing; the AGA/Cellulose ratio decreased to 0.40.

Table 69: Effect of freezing of tomato juice and concentrates on gross viscosity before and after dilution*.

| | before freezing | | | after freezing | | |
|---|---|---|---|---|---|---|
| | °Bx | %WIS | $\eta_{app}$ | °Bx | %WIS | $\eta_{app}$ |
| concentrates | 4.5 | 1.04 | 165.4 | 4.4 | 1.01 | 132.5 |
| | 8.0 | 1.82 | 652.8 | 7.9 | 1.77 | 568.5 |
| | 12.7 | 2.90 | 1543.0 | 12.6 | 2.82 | 1437.9 |
| | 18.4 | 4.22 | - | 18.4 | 4.03 | - |
| dilutions | 8.0 → 4.6 | 1.04 | 130.2 | 4.5 | 1.04 | 123.5 |
| | 12.7 → 4.5 | 1.04 | 122.1 | 4.4 | 0.99 | 109.8 |
| | 18.4 → 4.6 | 1.06 | 120.7 | 4.4 | 0.98 | 103.8 |

* Concentrates were obtained in a rotary vacuum evaporator on laboratory scale; an aliquot of the concentrates was frozen (-20°C) and stored for 6 weeks; measurements were done either directly on diluted non-frozen samples or on frozen samples which were diluted upon thawing. Juice of the variety H1361 was used.

Table 69 shows the effect of freezing on the gross viscosity of tomato juice and concentrates. Freezing the tomato juice caused a gross viscosity loss of 20%; concentrates also showed significant gross viscosity losses upon freezing. Freezing hardly increased the magnitude of the "dilution-loss" (unfrozen juice as reference). In this connection it should be noted that comparisons should only be made at corresponding WIS levels. It is obvious that frozen storage of both juice and concentrates affects the ability to detect a "dilution-loss".

## 7.4. *Conclusions*

The increase in the gross viscosity of tomato juices and tomato juice concentrates as a result of the use of hot break temperatures higher than those needed to inactivate the enzymes can be attributed to a better removal of WIS from the skin material. WIS "quality" and the microscopic appearence of the tomato cells are not affected by higher hot break temperatures. The effect of a specific hot break temperature on the removal of the WIS from the skin material probably depends on the tomato variety. The effects of hot break temperature, finisher temperature and finisher screen opening on the finisher operation are closely related to the effectiveness of the finisher.

The method of concentration or the type of evaporator used for concentration have no influence on the gross viscosity of concentrates. In general the WIS/TS ratio remains fairly constant during concentration.

A "dilution-loss" is observed when certain serum components (ions) are concentrated by evaporation in the presence of the tomato cells. The magnitude of the "dilution-loss" is influenced by mechanical shear, vacuum and heat processing of the concentrated juice. The quality of the juice before concentration also influences the "dilution-loss". The centrifugation-serum-concentration technique results in the prevention of the "dilution-loss" when a bench top centrifuge is used. The use of a decanter centrifuge in this technique results in a decreased "dilution-loss".

Both heat processing and freezing result in decreased gross viscosity of tomato juice and tomato juice concentrates.

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## *SAMENVATTING*

Het onderwerp en de doelstellingen van deze studie zijn nader uitgewerkt in hoofdstuk 1.

In hoofdstuk 2 wordt de relevante literatuur uitgebreid besproken. Dit literatuuroverzicht omvat de volgende onderwerpen: de produktie, algemene samenstelling en verwerking van tomaten (2.1.); definities en kwaliteitsparameters voor tomatenprodukten (2.2.); de faktoren die bijdragen aan de schijnbare viscositeit van tomatenprodukten (2.3.); de faktoren die de serumseparatie in tomatenprodukten beïnvloeden (2.4.) en de samenstelling van de polysacchariden in tomaten (2.5.). Gezien de lengte van 2.3. is een samenvatting van de, in deze paragraaf, besproken literatuur weergegeven in 2.3.1.

De algemene analytische methoden die meerdere keren tijdens het onderzoek zijn gebruikt worden besproken in hoofdstuk 3. In het onderzoek werd extra aandacht besteed aan een vergelijking tussen twee methoden van viscositeitsmeting en aan de bepaling van de neutrale suikers aanwezig in de wateronoplosbare bestanddelen (WIS) van tomatenprodukten.
De Bostwick consistometer bleek niet gevoelig genoeg voor het meten van hoog visceuse tomatenprodukten. Voor het meten van tomatenprodukten met een Bostwickwaarde groter dan 5 cm bleek dit instrument echter zeer geschikt. De hydrolyse van de polysacchariden in het WIS van tomatenprodukten met 0.8 N $H_2SO_4$ of 2 N $H_2SO_4$, beiden voorafgegaan door een hydrolyse met 72% $H_2SO_4$, resulteerde in een onbevredigende bepaling van de neutrale suikers. Hydrolyse met een mengsel van pektolytische en cellulolytische enzymen voorafgaand aan een hydrolyse met 2 N TFA leverde daarentegen goede resultaten op.

Het onderzoek naar de invloed van de oplosbare bestanddelen (serumviscositeit) en de onoplosbare bestanddelen op de schijnbare viscositeit van tomatensap en concentraten is beschreven in hoofdstuk 4. De schijnbare viscositeit van tomatensap en concentraten werd voornamelijk bepaald door het WIS gehalte. De invloed van de tomatenvariëteit, de "break" temperatuur en de methode van concentreren op de schijnbare viscositeit bij een bepaalde concentratie oplosbare bestanddelen (°Bx) kon geheel verklaard worden door verschillen in WIS gehalte. De schijnbare viscositeit bij een bepaalde WIS concentratie werd niet beïnvloed door de

tomatenvariëteit, de serumviscositeit, de "break"temperatuur en de methode van concentreren tenzij deze produktiemethoden leidden tot afbraak van het WIS.

De resultaten van het onderzoek naar de samenstelling en strukturele kenmerken van het WIS worden besproken in hoofdstuk 5. WIS fragmenten, in oplossing gebracht met behulp van extraktiemiddelen zoals ammoniumoxalaat, HCl en NaOH of met behulp van gezuiverde enzymen werden gekarakteriseerd d.m.v. gelpermeatie- en ionenwisselingschromatografie, analyse van de neutrale suikers en bepaling van de glycosidische bindingen. In het algemeen kon het WIS gefraktioneerd worden in 40-45% pektine, 25-30% hemicellulose en 30-35% cellulose. De pektinefraktie bevatte grote hoeveelheden galacturonzuur (AGA) en eiwit en bovendien ongeveer 25-35% van het arabinose en galactose aanwezig in het WIS. Het merendeel van dit arabinose en galactose bevond zich in de HCl oplosbare pektinefraktie en maakte deel uit van een sterk vertakt pektinesegment dat alleen via hoog veresterde AGA ketens aan de celwandmatrix gebonden was.
Een groot gedeelte van het pektine kwam voor in fragmenten die slechts in geringe mate vertakt waren met arabinose en galactose bevattende zijketens. De veresteringsgraad van deze fragmenten en de verdeling van de estergroepen was zodanig dat zowel PG als ook PL in staat waren deze fragmenten af te breken.
Xylanen, arabinanen en (arabino)galactanen bleken betrokken te zijn bij de binding van het pektine aan de celwandmatrix. De tomatenvariëteiten die bestudeerd werden verschilden vooral in het WIS gehalte en in de hoeveelheid arabinose en galactose aanwezig in het WIS. De verschillen in arabinose- en galactosegehalte kwamen het duidelijkst tot uiting in de HCl-oplosbare pektinefraktie. De samenstelling van deze pektinefraktie bleek sterk te veranderen tijdens de produktie van tomatensap. Het tomatenhemicellulose bevatte ongeveer 80% van het WIS-xylose. 60% van deze hoeveelheid was aanwezig in de vorm van β-1,4-xylanen, die waarschijnlijk verbonden waren met het pektine. 30% van de hemicellulose-xylose hoeveelheid bleek voor te komen in de vorm van fragmenten met een samenstelling kenmerkend voor xyloglucanen. Het hemicellulose bevatte verder ± 50% van het WIS-arabinose en WIS-galactose en bovendien 15-20% van het WIS-glucose. Alle arabinose en het merendeel van de galactose residuen bleken eindstandig in het hemicellulose voor te komen. De mogelijke

aanwezigheid in het hemicellulose van (gluco)mannanen en een geringe hoeveelheid galactanen werd aangegeven.
De cellulosefraktie bestond voornamelijk uit glucose, maar bevatte tevens ongeveer 15% van het WIS-arabinose.

In hoofdstuk 6 worden de resultaten weergegeven van het onderzoek naar de relatieve bijdrage van de WIS bestanddelen aan de schijnbare viscositeit van een tomatensapconcentraat en een verdunde tomatenpasta.
Commerciële en zuivere, goed gekarakteriseerde enzymen werden gebruikt om de schijnbare viscositeit van een verdunde tomatenpasta en een 10°Bx tomatensapconcentraat te verlagen.
De resultaten wezen, onafhankelijk van het gebruikte tomatenprodukt, op een belangrijke bijdrage van de hoger veresterde gedeelten in het WIS pektine aan de schijnbare viscositeit. Het werd waarschijnlijk geacht dat deze pektinefragmenten identiek waren aan de pektinefragmenten gevoelig voor afbraak door zowel PG als PL, zoals beschreven in hoofdstuk 5. De bijdrage van het laagveresterde pektine en het hemicellulose aan de schijnbare viscositeit bleef onduidelijk; de bijdrage van het eiwit was te verwaarlozen. Cellulose bleek een relatief kleinere bijdrage aan de schijnbare viscositeit te leveren dan het veresterde pektine. De starheid van de celwanden, veroorzaakt door de struktuur van cellulose scheen de schijnbare viscositeit bij hogere WIS gehaltes te beïnvloeden.
De conclusie werd getrokken dat de kleine verschillen in WIS samenstelling, zoals die van nature gevonden werden in de bestudeerde tomatenvariëteiten, geen aanleiding konden geven tot grote verschillen in de schijnbare viscositeit.
De schijnbare viscositeit van tomatensap en concentraten werd daarom voornamelijk bepaald door het WIS gehalte, dat waarschijnlijk evenredig is met de hoeveelheid tomatencellen.

De invloed van procesomstandigheden zoals: "hot break" temperatuur werd toegeschreven aan het effektiever verwijderen van het WIS van de schil. De kwaliteit van het WIS en het microscopisch uiterlijk van de tomatencellen werd niet beïnvloed door hogere hot break temperaturen.
De invloed op het passeren van de "hot break" temperatuur, de temperatuur tijdens het passeren en de diameter van de openingen in de passeerzeef, hing nauw samen met de effectiviteit van de passeermachine.
De schijnbare viscositeit van concentraten van tomatensap bleek niet

beïnvloed te worden door de methode van concentreren noch door het type verdamper dat gebruikt werd. In het algemeen veranderde de verhouding WIS/TS nauwelijks tijdens het concentreren. Het gelijktijdig concentreren van bepaalde serumcomponenten (waarschijnlijk ionen) en tomatencellen tijdens het verdampen bleek tot een viscositeitsverlies te leiden na verdunning van de pasta ("dilution-loss"). De grootte van het "dilution-loss" werd beïnvloed door mechanische krachten en vacuum tijdens het verdampen en door een hittebehandeling van het geconcentreerde sap. De "hot break" temperatuur en de verdamptemperatuur bleken geen invloed op de grootte van het "dilution-loss" te hebben. Het "dilution-loss" bleek tevens beïnvloed te worden door de kwaliteit van het sap voorafgaand aan het concentreren. Het concentreren van tomatensap met behulp van de "centrifugeren serum concentreren" methode voorkwam het "dilution-loss" wanneer gebruik werd gemaakt van een tafelcentrifuge en reduceerde het "dilution-loss" wanneer gebruik werd gemaakt van een decanter.
De schijnbare viscositeit van tomatensap en concentraten werd nadelig beïnvloed zowel door een conserverende hittebehandeling als ook door bevriezing.