Physico-chemical stability of tomato products

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F.W.C. den Ouden

Physico-chemical stability of tomato products

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
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Stellingen

1. De mate van ordening en de fysische staat van het cellulosenetwerk in de celwanden van plantecellen is bepalend voor de viscositeitsverandering bij het homogeniseren van plantecelsuspensies.
Dit proefschrift, hoofdstuk 4.

2. Homogenisatie van tomatecelsuspensies leidt tot een toename van het elastische karakter.
Dit proefschrift, hoofdstuk 4.

3. Enzymatische hydrolyse van geconcentreerde tomatecelsuspensies met polysaccharide afbrekende enzymen bevordert serumseparatie.
Dit proefschrift, hoofdstuk 5 en 6.

4. Het "plakken" van tomatecelsuspensies aan verpakkingsmateriaal, verhoogt de stabiliteit tegen serumseparatie.
Dit proefschrift, hoofdstuk 7.

5. Microscopische breuk van het microfibrillaire cellulosenetwerk speelt een essentiële rol bij de viscositeitsverliezen die optreden door het concentreren en terugverdunnen van een tomatecelsuspensie.
Dit proefschrift, hoofdstuk 2.

6. De door Stoforos en Reid ontwikkelde methode om serumvorming in tomatenketchup te meten is eigenlijk een permeabiliteitsmeting en geen bepaling van de serumvorming.
Stoforos and Reid, 1990. J. of Food Sci. 55, 1626-1629.

7. De aanduiding "hairy region" voor het rhamnoserijke gedeelte van het pektine-molekuul zou beter vervangen kunnen worden door "branched region" of "ramified region".
Schols et al, 1990. Carbohydr. Res., 206, 117-129.

8 Niet gemodificeerd sojaeiwit is ongeschikt als basis voor biologisch afbreekbaar plastic.

9. Extractiemethoden om het gehalte aan actief stremsel in kaas te bepalen, zijn vanwege hun lage recovery zeer aanvechtbaar.

10. De uitspraak van Umberto Eco dat creativiteit niet gelegen is in het vinden van nieuw materiaal, maar in herschikking van het bestaande, is niet geheel juist.

Umberto Eco, De volkskrant 16 januari 1995.

11. In de milieudiscussies over verpakkingsafval krijgt meermalig verpakkingsglas onevenredig veel aandacht.

Milieuplan Glas verpakkingen Branche Vereniging Glas.

12. Tomatenpuree heeft uitstekende vetvervangende eigenschappen.

13. Het moment bepaalt of de balans doorslaat.

Stellingen bij het proefschrift "Physico-chemical stability of tomato products" van F.W.C. den Ouden te verdedigen op 6 juni 1995 te Wageningen.

ter nagedachtenis aan mijn vader

voor Anita

Abstract

Ouden, den, F.W.C. (1995). Physico-chemical stability of tomato products. Ph.D. thesis, Wageningen Agricultural University (pp. 113, English and Dutch summaries).

Key words: cell wall, enzymes, homogenization, pectin esterase, rheology, tomato.

The effect of some physical processes and enzymatic hydrolysis on the physico-chemical properties of tomato suspensions was studied.

Concentration degree has a large effect on the apparent viscosity and the storage modulus of suspensions after being diluted to a standardized water insoluble solids level. Besides decrease in average particle size, microscopic fracture of the cellulosic microfibrillar network during concentration are thought to be responsible for this phenomenon. By wet sieving it was shown that the bulk of the particles has a size between 45-180 μm. The tomato cell wall seems to be highly deformable. The 90-180 μm wet sieve fraction had highest apparent viscosity and yield stress as well before as after homogenization.
Homogenization of tomato suspensions as well as of strawberry sauce led to an increase in the apparent viscosity and storage modulus, whereas that of apple sauce led to a decrease in the apparent viscosity. The difference in behaviour upon homogenization is due to a difference in fracture behaviour of the plant cells, which is probably a result of the cell wall structure, especially the microfibrillar cellulose structure.

Incubation of tomato suspensions with highly purified well specified polysaccharide degrading enzymes resulted in a decrease in rheological parameters. By homogenization the apparent viscosity increased to higher values compared to that of the non enzyme treated tomato suspension. The enzyme preparations gave rise to more serum separation. Hydrolysis of diluted hot break paste by pectin esterase from oranges or fungi resulted in a much higher yield stress, which is probably due to the formation of a calcium pectinate network. The apparent viscosity became only slightly higher. Serum viscosity increased initially, after which it decreased to about 50% of the original value.

The main physical problem of tomato suspensions is the formation of a serum layer on top of it. Several mechanisms are responsible: uniaxial compression of the weak particle network due the gravitational force and drainage of serum as a result of unevenness in the surface. The physical behaviour of tomato suspensions can be better understood by considering the tomato cell wall as a concentrated, composite gel consisting of cellulose microfibrils embedded in a "jelly" matrix of pectic and hemicellulosic substances.

Voorwoord

Aan de totstandkoming van dit proefschrift hebben veel mensen, uit diverse disciplines, een bijdrage geleverd, waarvoor ik ze hartelijk wil bedanken.

Op de eerste plaats bedank ik mijn moeder die mij altijd de vrijheid en de mogelijkheid gegeven heeft om te studeren.

I am very grateful to the H.J. Heinz company for funding this project. Especially I would like to thank Tineke van Vliet and Dick van Olderen for taking the initiative and offering this challenge, and for their enthousiastic and motivating contribution to this research project. I am also much indebted to the TTOC members for their ideas and the stimulating discussions.

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Henk van der Stege, Henk Jansen, Wim Lammers, Hugo Stempher, Aliza de Groot van de sectie Zuivel en Levensmiddelennatuurkunde en Ben van den Broek van de sectie Levensmiddelenchemie wil ik bedanken voor de technische ondersteuning bij dit onderzoek. John van Bolderen van Heinz bedank ik voor het regelmatig verrichten van analyses.

In het kader van hun studie hebben de volgende personen een bijdrage geleverd: Baukje Tinga, Martin Stigloher, Michael Mensah Wilson, Ron Grotenhuis, Arjan Pitlo en Johanna Nink; allemaal bedankt voor jullie inzet.

Andre de Roos, waarmee ik enkele jaren een kamer gedeeld heb, bedank ik voor de discussies en de gezelligheid. William Kloek ben ik dank verschuldigd voor zijn ideeën en zijn praktische tips.

I would like to thank Mr Tony Skrimshire from Heinz for reading the manuscript of this thesis.

Tevens bedank ik een ieder van de sectie Zuivel en Levensmiddelennatuurkunde voor de gezelligheid en de zeer prettige sfeer waarin ik dit onderzoek heb kunnen verrichten.

Tot slot, Anita, door jou is deze periode wel heel bijzonder geworden, aan jou draag ik dit proefschrift op.

Contents

1 Introduction

1.1 Tomato products; compositional features

Tomato products are important to human food consumption. The largest part of the world tomato crop is processed into tomato paste, which is used as an ingredient in many products such as soups, sauces and ketchup. An example of a flow sheet of a tomato paste process is given below.

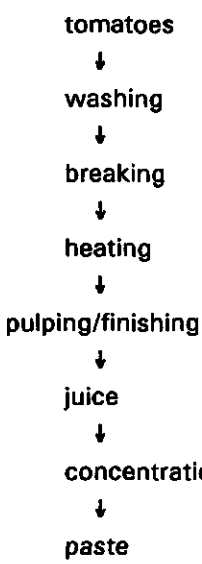

Figure 1. Example of a flow sheet of a tomato paste process.

Tomato paste can be produced either by the cold break or by the hot break process. In a hot break process the crushed tomatoes are immediately heated to a temperature of at least 90 °C, by which the pectolytic enzymes, present in the tomatoes are inactivated. In a cold break process the crushed tomatoes are heated to temperatures of 40-60 °C. Then the pectolytic enzymes retain a large part of their activity and are able to degrade the cell wall pectin during subsequent processing. During pulping/finishing the crushed and heated tomatoes pass a series of sieves with pore sizes ranging from 1.5 to 0.5 mm; skins and seeds are removed. Subsequently, the resultant juice is concentrated, often in a three stage evaporation process, to tomato paste with a refractive index of e.g. 28-30 °Brix.

The composition of tomatoes depends on many factors e.g. cultivar and ripening stage (Luh et al, 1954, 1984). In addition there are differences in

composition between different parts of the fruit (Buescher et al, 1979). The dry matter of tomatoes generally lies between 5 and 7.5 %. Of this dry matter about half consists of reducing sugars, with slightly more D-fructose than D-glucose (Davies and Hobson, 1981; Williams and Bevenue, 1954). The water insoluble solids (WIS) content of tomato juice lies between 0.7 and 1.1% (Heutink, 1986, Stevens and Paulson, 1976). In fact water unextractable solids would be a better name, but in this thesis the conventional name is used. The largest part (circa 75%) of the WIS comes from the cell walls (Lindner et al, 1984). The other 25% of the insoluble solids consists of extracellular protein rich granules. The main constituents of tomato cell walls are pectic substances, hemicelluloses, cellulose and protein (Davies and Hobson, 1981). According to Heutink tomato WIS consists of 40-45% pectin, 25-30% hemicellulose and 30-35% cellulose. The largest part of the protein, making up about 20% of the WIS, was found in the pectin fraction. Pectic substances in tomato fruit are mainly composed of galacturonic acid with small amounts of galactose, arabinose and rhamnose, but xylose, ribose, glucose and mannose have also been reported (Davies and Hobson, 1981; Heutink, 1986). Galactose, arabinose and rhamnose are part of a highly branched pectin segment (Heutink, 1986). According to Seymour et al (1990) side chains of pectic polysaccharides consist of ß-(1-4)-linked galactopyranosyl and α-(1-5)-linked arabinofuranosyl residues. Pectins are partly bound by non covalent bonds (Fishman et al, 1989). Classical hemicellulose fractions of plant cell walls consist of xylans, arabinoxylans, mannans, glucomannans and galactomannans (Gross and Wallner, 1979). The tomato hemicellulose contains approximately 80% of the WIS xylose of which 60% is present in β-(1-4)-xylans. Also xyloglucans have been identified in tomato cell walls (Heutink, 1986). The arabinose and part of the galactose of the hemicellulose are present as terminal residues. According to Seymour et al (1990) the major hemicellulosic polysaccharide is a xyloglucomannan.

The sugar composition (%W/W) of the polysaccharides from the WIS fraction of hot break juice is given in Table 1 (Heutink, 1986). As can be seen from Table 1 the most important sugars with respect to quantity are glucose and anhydrogalacturonic acid. Largest part of the glucose occurs in the cellulose fraction as β-(1-4)-glucan, whereas anhydrogalacturonic acid is the main constituent of pectin.

Varieties especially differ in rhamnose, arabinose, galactose and mannose content (Heutink, 1986). Besides, pectic fractions of tomato varieties may differ in average molecular weight (Luh et al, 1984).

During ripening of the tomato, the composition of the cell wall changes; galacturonic acid, galactose and arabinose decrease in quantity (Gross and Wallner, 1979).

Table 1. Sugar composition in W/W % (expressed as ranges between the extremes) of the polysaccharides from the WIS fractions of hot break juice from 4 tomato varieties (Heutink, 1986).

Sugar	% W/W
glucose	45.8 - 55.0
anhydrogalacturonic acid	23.0 - 27.8
mannose	5.2 - 11.1
xylose	5.7 - 7.5
galactose	4.9 - 6.8
arabinose	2.1 - 4.6
rhamnose	0.3 - 1.7

Ripe tomatoes contain much less galactan side chains compared to unripe tomatoes (Seymour et al, 1990). The hemicellulose fraction of the unripe and ripe fruit shows negligible difference in composition (Gross and Wallner, 1979; Seymour et al, 1990; Wallner and Bloom, 1977). The concentration of xylose, non cellulosic glucose, mannose, cellulose and protein was found not to be affected by ripening (Gross and Wallner, 1979).

Besides composition of the cell wall, the way in which the polysaccharides are organized into the tomato cell wall, is important for its physico-chemical properties. Many models for plant cell walls have been proposed in the past (Keegstra et al, 1973; Robinson, 1977; Lamport and Epstein, 1983; Albersheim, 1975). It goes far beyond the scope of this thesis to give a complete treatise of all these models. Recently a model for the cell wall has been proposed by Carpita and Gibeaut (1993) (Fig. 2). According to this model the cell wall is a network of cellulose microfibrils consisting of chains of β-(1-4)-linked D-glucose. These microfibrils are 5-15 nm thick and spaced 20-40 nm apart (McCann and Roberts, 1992). The xyloglucans connect the cellulose fibrils. Also other non cellulose polysaccharides are able to interlock the microfibrils such as glucomannans and galactoglucomannans. Xyloglucans are linear chains of β-D-(1-4)-glucan with xylosyl units attached at regular sites to the *O*-6 position of the glucosyl units of the chain. Additional sugars, β-D-galactose and α-L-arabinose are added to the *O*-2 of some xylosyl units (Bacic et al, 1988). Only one side of the linear xyloglucan backbone is able to bind tightly to the surface of cellulose microfibrils. Extracted xyloglucans are 20-700 nm long with most being about 200 nm. According to Carpita and Gibeaut (1993) the cell wall comprises three fractions: the fundamental cellulose-xyloglucan frame work which is embedded in a second fraction of pectic polysaccharides with a "jelly character". The third fraction consists of the structural proteins, which strengthen the cell wall (Cassab and Varner, 1988).

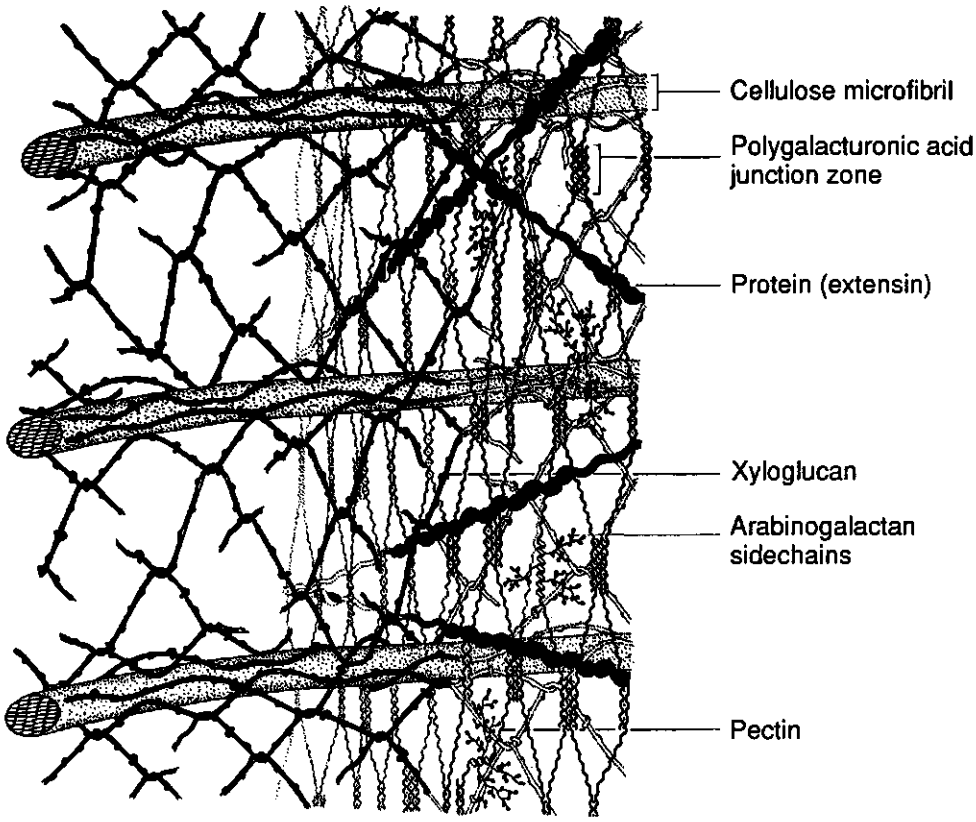

Figure 2. Structural model of primary cell wall as proposed by Carpita and Gibeaut (1993).

The consequences of cell wall structure for technological purposes can be better understood if the biological functions of the cell wall components are known. The cellulose fibrils are presumed to form the skeleton, they give strength to the cell wall. Therefore cellulose is an important constituent determining the physical properties of tomato products. The pectin fraction has many biological functions; they determine the pore size of the walls (Baron-Epel et al, 1988), which is important for the diffusion of enzymes and therewith for the biochemical processes in the cell. Pore size is affected by location and size of the deesterified blocks in the pectin chain and by the size and conformation of the pectin side chains. According to some investigators the branched parts of the pectin molecules also act as signal molecules for enzymes involved in cell or cell wall metabolism (Carpita and Gibeaut, 1993).

The activity of many enzymes in tomato fruit has been demonstrated (Davies and Hobson, 1981). The most important enzymes involved in ripening are pectin

esterase (PE) and polygalacturonase (Besford and Hobson, 1972). The action of these pectic enzymes influences the consistency of tomato products (Garces and Luh, 1972; Lee and MacMillan, 1968; Luh et al, 1954, 1956, 1960; Robinson et al, 1956). Tomato pectin esterase, like most other plant pectin esterases, deesterifies pectin linearly along the pectin molecule, creating blocks of free carboxyl groups. The enzyme starts at reducing ends of highly esterified pectin molecules (Lee and Macmillan, 1970) and at the methylester groups next to free carboxyl groups (Kohn et al, 1968 and 1983). After deesterification of pectin by PE, the pectin molecule becomes very susceptible to hydrolysis by polygalacturonase (Goodenough, 1981; Pressey and Avants, 1982). In tomato fruit both exo- and endo-polygalacturonase have been demonstrated (Goodenough, 1981). Polygalacturonase is absent in green tomatoes, however, its concentration increases strongly during ripening (Hobson, 1964; Gross and Wallner, 1979; Tucker et al, 1980). Tomato pectin esterase is inactivated by heating at 82 °C for 15 sec, endo-polygalacturonase is much more heat stable; it is inactivated by heating at 104 °C for 15 sec (Garces and Luh, 1972; Luh and Daoud, 1971). Polysaccharide degrading enzymes others than polygalacturonase and pectin esterase which have been found in tomato fruit are e.g. xyloglucan endo-transglycosidase (Maclachan and Brady, 1994), ß-galactosidase (Pressey, 1983) and ß-glucanase (Maclachlan and Brady, 1992; Wallner and Walker, 1975; Phar and Dickinson, 1973).

1.2 Rheological aspects of tomato products

The apparent viscosity is an important quality attribute of tomato products. In concentrated tomato products like tomato paste, the apparent viscosity is primarily determined by the concentration of cell wall material expressed as water insoluble solids (Tanglertpaibul and Rao, 1987; Heutink 1986). The apparent viscosity of tomato concentrates was found to be proportional to the 2.5 power of the concentration of total solids (Rao et al, 1981). In tomato products with a relatively low WIS content (approximately 1%) like tomato juice, the apparent viscosity is also determined by the physico-chemical characteristics of the WIS particles (Whittenberger and Nutting, 1957 and 1958) and by the serum viscosity (Marsh et al, 1980). The water soluble high molecular weight polymeric compounds contribute to a lesser extent to the apparent viscosity than the percentage of WIS (Marsh et al, 1980).

Serum of tomato juice behaves as a Newtonian liquid (Tanglertpaibul and Rao, 1987). At a high concentration of dry matter serum becomes non Newtonian (Harper and Sahrigi, 1965), which is probably due to soluble high molecular pectic substances. Tomato concentrates exhibit shear thinning (Harper and

Sahrigi, 1965; Tanglertpaibul and Rao, 1987), and thixotropic behaviour and have a yield stress (Jiminez et al, 1989). Mathematical models have been used to describe the flow behaviour of tomato concentrate (Rao, 1987). According to Rao et al (1981) flow data of tomato concentrates can be described satisfactorily by the power law and the Casson model (Whorlow, 1992). The power law model gave higher correlation coefficients. The Herschel-Bulkley model, with as yield stress the Casson yield stress, also described the data satisfactory, but correlation coefficients were lower than for the simple power law.

Several studies have been conducted to establish the relative contribution of various constituents (pectin, cellulose) of the tomato cell to the apparent viscosity by the use of enzymes. Care should be taken with the interpretation of the results of these studies because the enzyme preparations used were not always pure, they exhibited some contaminating activities. Secondly, it is very difficult to remove one constituent without disturbing the structure of the remaining cell wall. Cellulose as well as pectin seem to be important for the apparent viscosity of tomato juice (Foda and McCollum, 1970; Heutink, 1986; Whittenberger and Nutting, 1957). Degradation of proteins of tomato juice with proteinase caused a relatively small decrease in apparent viscosity (Foda and McCollum, 1970; Heutink, 1986).

Numerous articles have been written in which apparent viscosity and other physical properties of tomato juice and tomato concentrate have been related to processing conditions like heating, finishing, concentration and homogenization (Heutink, 1986). In fact these processing conditions have an indirect effect on the apparent viscosity, by influencing physico-chemical characteristics of the WIS particles, WIS concentration and serum viscosity.

The rheological properties of tomato juice and tomato paste are strongly influenced by the heating procedure during breaking of the tomatoes. Apparent viscosity as well as serum viscosity are higher as break temperature is higher (Luh and Daoud, 1971; Molwane and Gunjal, 1985). Xu et al (1986) studied rheological properties of juice and paste of various cultivars at breaking temperatures of 85, 96 and 107 °C. The apparent viscosity of tomato juice and paste varied with cultivar and breaking temperature. Highest viscosity was obtained after a breaking temperature of 107 °C. This is probably caused by the very high heat resistance of polygalacturonase, it is only inactivated by a heat treatment of 15 sec at 104 °C. The high viscosity of hot break tomato juice is related to its pectin content and the physico-chemical nature of the pectin. In hot break juice more of the higher molecular weight fraction is retained than in cold break juice. The methoxyl content of hot break juice pectin was found to be higher than in cold break juice (Bhasin and Bains, 1987; Heutink, 1986).

Care must be taken in interpreting effects of heating on apparent viscosity of the tomato products (Crandall and Nelson, 1975). During heating two reactions take place. At first the enzymes present in the tomato, mainly pectolytic enzymes, are inactivated. Secondly, heating causes hydrolysis of the pectic substances. Caradec and Nelson (1985) exposed tomato juice to temperatures of 82, 102 and 112 °C. Heat temperature affected serum viscosity. A heat treatment of the juice at 82 °C applied for 2 h resulted in 17-30% loss of serum viscosity, whereas a treatment at 112 °C for 2 h caused 67-82% loss. These experiments illustrate significant changes in rheological properties due to chemical hydrolysis which may play a part during processing of tomatoes to tomato paste.

Dynamic rheological measurements

In this research dynamic rheological measurements have been used to characterize the viscoelastic structure of tomato suspensions; the parameters obtained by these measurements give information about the network structure of the product. In dynamic rheological measurements both the shear stress and the shear strain vary in time (Whorlow, 1992). Usually they are varied according to a sinusoidal pattern at an angular frequency ω. In an apparatus like the Bohlin VOR the shear strain varies in time whereas the associated sinusoidally varying shear stress is monitored. In case of a linear viscoelastic sample, the stress amplitude is proportional to the strain amplitude and oscillates at the same frequency but out of phase (phase angle: δ) with the strain (Fig. 3).

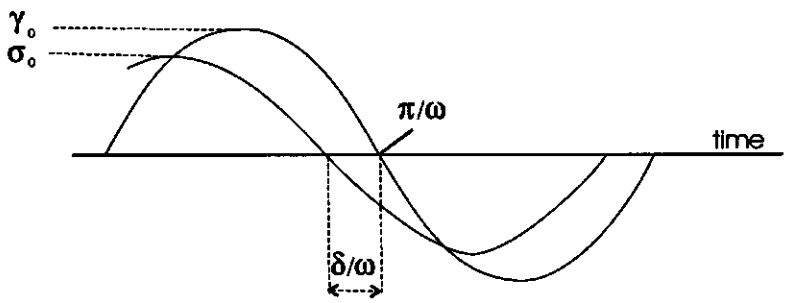

Figure 3. Shear strain (γ) and shear stress (σ) as a function of time in a dynamic rheological measurement for a viscoelastic material.

The shear strain (γ) and shear stress (σ) are given by

$$\gamma(t) = \gamma_o \sin(\omega t) \quad (1)$$

$$\sigma(t) = \sigma_o \cos(\omega t + \delta) = \sigma_o(\sin(\omega t)\cos\delta + \cos(\omega t)\sin\delta) \quad (2)$$

where γ_o is the maximum shear strain, σ_o the maximum shear stress and δ is the phase angle between strain and stress. For an ideally elastic solid $\delta = 0$ °, for a pure viscous fluid $\delta = 90$ °. For viscoelastic materials δ lies between 0 and 90°. From the amplitude difference and the phase shift, the storage modulus G', the loss modulus G'' and tan δ can be calculated (Whorlow, 1992).

$$G' = (\sigma_o/\gamma_o)\cos\delta \quad (3)$$

$$G'' = (\sigma_o/\gamma_o)\sin\delta \quad (4)$$

$$\tan\delta = G''/G' \quad (5)$$

G' is related to the elastic contribution of the material to an applied stress or strain, in fact it is a measure of the energy storage in a material during a periodic application of a stress or strain. G'' is associated with the viscous contribution of the material to an applied stress or strain; it is a measure of the energy dissipation in a material during a cycle of stress or strain. Tan δ is a measure of the ratio between the viscous and elastic contribution. A higher tan δ means in general that the product behaves more viscous.

1.3 Objectives and outline of the thesis

When tomatoes are processed into tomato juice, tomato paste or other tomato based products, the tomato suspensions are subjected to physical processes such as heating, finishing, concentration and homogenization. Due to these processes physical, chemical and enzymatic changes occur in the tomato suspensions. Although a lot of studies have been conducted on these issues, there is still lack of fundamental knowledge about the changes in the physico-chemical properties of tomato suspensions, particularly that of the cell wall constituents, during the processes mentioned above.

The objective of this thesis was to obtain fundamental knowledge about the physico-chemical changes that occur in tomato suspensions due to some of these processes.

In chapter 2 some general rheological techniques are presented which have been applied to characterize the physico-chemical properties of tomato suspensions. The results of the study on the effect of concentration on the physico-chemical properties are also presented.

In chapter 3 some methods for determining the particle size distribution of tomato products are discussed, besides, the effect of particle size on some rheological properties is given.

Chapter 4 describes the results of the study on the effect of homogenization of tomato suspensions. In order to get a better understanding of the mechanisms of this process also the effect of homogenization on other fruit products (apple sauce and strawberry sauce) was investigated.

Chapter 5 and 6 both deal with enzymatic modifications of tomato suspensions. In chapter 5 the results of hydrolysis by some polysaccharide degrading enzymes are presented. In chapter 6 the effect of pectin esterase from oranges or from fungi, on the physico-chemical properties is given.

Chapter 7 has been devoted to the physical stability of tomato products against serum separation.

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2 Rheology of tomato products; Effect of concentration

Summary

Measuring methods for apparent viscosity, yield stress and dynamic moduli of tomato suspensions were established. Overshoot experiments at low shear rates proved to be a good method for estimating "yield stresses". The °Brix value of the tomato concentrate, from which tomato suspensions were prepared, was shown to have a large effect on its apparent viscosity and storage modulus. The apparent viscosity of a tomato suspension prepared from a 30 °Brix tomato concentrate was only 35% of that of a suspension prepared from a 4.9 °Brix juice, both standardized on the same water insoluble solids (WIS) and total tomato solids (TS) level. After homogenizing the difference in apparent viscosity was only ca 10-15%. A similar trend was found for the dynamic moduli. The decrease in rheological parameters of the non homogenized suspensions is probably related to a decrease in diameter of the tomato particles due to the concentration process. Moreover, microscopic fracture of the cellulosic, microfibrillar network of the cell wall may play a part. Serum separation of diluted non homogenized tomato suspensions was higher if made from a concentrate that was concentrated to a higher °Brix value. This phenomenon is discussed in terms of variations in uniaxial compression of the network in the diluted tomato suspensions due to gravitational force.

2.1 Introduction

Consistency is an important quality attribute of tomato products like tomato juice, tomato paste and tomato sauce (Gould, 1992). Here, consistency is used in a more general meaning than the official one, denoting the whole of rheological properties thus including a parameter such as yield stress. In literature, many ways of expressing consistency are used; (apparent) viscosity, gross viscosity, Bostwick consistency, Adams consistency. The US Department of Agriculture (USDA) expresses consistency of tomato sauce and tomato ketchup as the distance in cm that the product flows in 30 sec at 20 °C in the Bostwick consistometer (Davis et al, 1954). This measuring method is empirical; obtained results cannot be translated to fundamental physical parameters as apparent viscosity or yield stress. Therefore measurements with well designed rheometers which allow the determination of fundamental parameters are preferred more and more.

Concentration of tomato juice effects the physical properties of the end product. Although new technologies have been introduced, e.g. reverse osmosis (Merlo et al, 1986), and freeze concentration (Deshpande et al, 1984), concentration by evaporation is still the most conventional technology.

Mannheim and Kopelman (1964) studied the effect of two methods of evaporation on the consistency of the end product; whole juice evaporation and serum evaporation after separation of the pulp by centrifugation. In the latter case the final concentrate was obtained by combining the concentrated serum and pulp obtained by centrifugation. The juice was prepared by breaking the tomatoes at 60 °C (= cold break) and holding for 5 minutes at that temperature. The concentrate made by the first method had a higher viscosity, although serum evaporation involved shorter evaporation times. According to them, the difference in viscosity might be attributed to the centrifugation step during the separation of the cold break juice which led to crushing of cells and so to disruption of the solidlike suspension structure of the resulting juice. However, it is also possible that the pectolytic enzymes might have had a negative effect on the consistency. Restoration of the solidlike structure, if at all possible, could not be achieved by ordinary re-mixing of the separated pulp and serum.

Tanglertpaibul and Rao (1987) found that apparent viscosities of concentrates made by evaporation of hot break tomato juice were lower than those obtained by centrifugation followed by evaporation or reverse osmosis of the separated serum and remixing. This result indicates that the results of Mannheim and Kopelman (1964) are affected by the action of enzymes in the cold break paste.

Harper and Sahrigi (1965) determined the viscosity of 16% and 25% total solids concentrates prepared by dilution from a 30% concentrate of a hot break juice and compared the resulting data with those of the original concentrates of 16 and 25%. In both cases they observed a lower apparent viscosity compared to the original concentrates of 30 and 20% respectively. The authors ascribed this result to experimental errors.

Heutink (1986) found a difference between apparent viscosity of juice and concentrates on the one hand and apparent viscosity of diluted paste at the same solids level on the other hand. He defined the decrease in viscosity as "dilution loss". A "dilution loss" of 65-70% was found when tomato juice (5 °Brix) was concentrated to 29 °Brix and diluted back to the original concentration. The temperature of evaporation seemed to have no influence. According to Heutink (1986) the magnitude of the "dilution loss" depended strongly on the method of concentration. "Dilution loss" was also effected by the solids content of the concentrate. It was found to be proportional to the

amount of water evaporated; additional heating of the diluted samples for 15 min in boiling water resulted in a small gross viscosity increase but it did not negate the "dilution loss". The explanation given was that collapsed cells, a consequence of the concentration process, are not able to reabsorb water and to expand to the original state.

The aim of this study was to establish reliable methods to measure rheological properties like apparent viscosity , yield stress and dynamic moduli of tomato suspensions. Besides the effect of concentrating the tomato juice by evaporation on some of these properties was studied. Because in practice several tomato products are homogenized during manufacturing, rheological properties were determined before and after homogenization of the tomato suspensions.

2.2 Experimental

Preparation of a standard tomato suspension

A standard tomato suspension was prepared containing 30% tomato paste (28 °Brix). The suspension was standardized at 0.58 M NaCl, pH 3.4 and 32.7 °Brix, by mixing the ingredients (tomato paste, NaCl, saccharose, acetic acid and water) and heating to 90 °C. Subsequently the mixture was homogenized at 17 MPa/90 °C (Rannie, Denmark) and cooled to about 25 °C.

Water insoluble solids (WIS) and total tomato solids (TS)

For the determination of WIS circa 20 gram of tomato product was centrifuged in a Sorval RC-5B at 30.000 *g* for 20 min. After decanting the supernatant, the residue was washed with water (circa 70 gram) at room temperature. This was repeated 4 times, then the supernatant had a refractive index of about 0 °Brix. The residue was dried in an oven at a temperature of 110 °C (16 h), weighed and the result expressed as % WIS.

The percentage of TS of tomato concentrate was determined using a vacuum oven at a temperature of 70 °C (2 h). The product was mixed with seesand.

Preparation of tomato concentrates of varying °Brix

The term tomato concentrate in this thesis is used for products obtained by concentration of tomato juice, independently of the °Brix value of the end product. Tomato paste should contain at least 24% natural tomato soluble solids as measured on the °Brix scale of a refractometer (Lamb, 1977).

Tomato concentrates of about 10, 15, 20, 25 and 30 °Brix were prepared by evaporation of an industrially, manufactured hot break juice using a pilot plant evaporator (Rossi Catelli, 40 l) at a temperature of about 65-70 °C and an

underpressure of about $9.0\text{-}9.5 \cdot 10^4$ Pa. The composition of the concentrates is given in Table 1. From these concentrates, suspensions with a concentration of 0.65% tomato WIS, 32.1 °Brix, 0.58 M NaCl and pH 3.4 were prepared by mixing the ingredients (tomato concentrate, NaCl, acetic acid, saccharose, water) at room temperature (22 °C). The products were homogenized at 8 MPa and 22 °C with a Rannie lab homogenizer (Minilab, 8.30H).

Table 1. Composition of concentrates of varying °Brix.

°Brix	pH	Total Solids (%)	WIS (%)	WIS/TS
4.9	4.65	5.66	0.96	0.17
10.0	4.73	11.38	2.10	0.18
16.8	4.69	18.41	3.27	0.18
19.3	4.65	21.07	3.44	0.16
24.8	4.65	25.51	4.67	0.18
30.5	4.59	31.93	5.64	0.18

Laser Diffraction

A rough estimate of the size distribution of the particles was obtained by a laser diffraction technique using a Coulter LS 130 system. In this instrument laser light (wavelength 720 nm) is scattered by the particles, and the generated diffraction pattern, which is a composite of the individual diffraction patterns of all the particles, is measured. The instrument converts this composite diffraction pattern to a particle size distribution with 72 classes ranging from 0.3 to 900 μm, by using the Fraunhofer theory.

Rheological measurements

Apparent viscosities (η^*) were determined using either a Bohlin VOR Rheometer fitted with sherated plates (diameter 3 cm) or a Haake Rotovisco RV20. In order to prevent slip the last one was fitted with a coaxial cylinder measuring system, type P (ribbed outer cylinder) and a MV3 inner cylinder covered with sandpaper (cornsize 80, 0.02-0.04 mm). To check the effect of slip some experiments were done with a smooth MV/MV3 measuring system. Before starting a measurement, the product was stirred several times with a spoon and then poured carefully in the measuring system of the rheometer. An equilibration time of 15 minutes was taken into account in order to diminish possible thixotropic effects. Measurements were started at the lowest shear rate ($\dot{\gamma}$) followed by increasing

shear rates. At each shear rate the shear stress (σ) was determined after 3 min of shearing.

The yield stress (σ_y) was determined using a constant stress Deer PDR 81 Rheometer, fitted with parallel plates (5 cm), both covered with sandpaper and a distance between the plates of 2 mm. A constant stress was applied and the shear strain was measured as a function of time. The minimum stress at which a viscous component ($\eta^* < 10^3$ Pas) was observed, was taken as the yield stress. Because these experiments are very time consuming a second method, for estimating the yield stress, was established using so-called overshoot experiments with a Haake Rotovisco rheometer. In these experiments σ was determined as a function of time in an experiment at low $\dot{\gamma}$ (0.024 s^{-1}). The maximum σ was taken as a measure of the yield stress.

Dynamic experiments were carried out with a Bohlin VOR rheometer, fitted with sherated plates (diameter 3 cm). During these experiments shear stress, shear strain and the shear rate vary in time in a sinusoidal way with an angular frequency. From these measurements G', G'' and tan δ can be calculated (Whorlow, 1992). G', the storage modulus, is a measure of the energy stored and next released during one cycle of sinusoidal varying strain; G'', the loss modulus, is a measure of energy dissipated as heat per cycle of deformation. The third parameter tan δ (G''/G') is a measure of the ratio between the viscous and elastic component in the mechanical response of the material on an applied stress or strain.

2.3 Results and discussion

2.3.1 Some rheological properties of tomato suspensions

When determining rheological properties of dispersed food products with relatively large particles, slip is often observed (Qiu and Rao, 1989; Grikshtas and Rao, 1993). The occurence of slip for a homogenized tomato suspension was checked by determining its apparent viscosity in two ways: (1) in a Haake Rotovisko fitted with the profiled outer cylinder (P) and inner cylinder MV3, covered with sandpaper and (2) the same instrument fitted with a smooth coaxial cylinder system (MV/MV3).

As can be seen from Fig. 1 the determined apparent viscosity (η^*) is significantly lower for the system with the smooth cylinder surfaces. This was due to a kind of slip phenomena which may be caused by the fact that near the surface of the measuring body the concentration of particles is lower due to excluded volume effects and therewith the apparent viscosity. Moreover, some serum separation near the wall of the measuring body may occur, also resulting in a layer with a low apparent viscosity. Therefore all subsequent rheological

measurements were done with sandpaper covered or roughened measuring bodies.

As a tomato suspension is a viscoelastic fluid, depending on the stress, three regions can be established: pure elastic deformation at very low shear stresses; viscoelastic deformation at intermediate stresses, the product behaves elastic and viscous at the same time. At still larger stresses the product behaves viscous, elastic effects may be neglected. Yield stresses were determined by doing creep experiments at various stresses. The stress at which a sudden increase in shear rate was observed was taken as the yield stress. The value differs from the real yield stress which is the stress where the product changes from elastic behaviour, into viscoelastic behaviour.

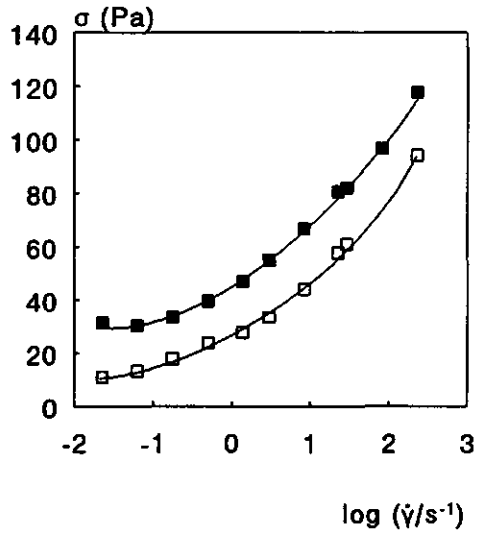

Figure 1. Shear stress (σ) as a function of shear rate ($\dot{\gamma}$) for a tomato suspension (30% tomato paste) homogenized at 17 MPa, as measured with a Haake rheometer fitted with the MV/MV3 (□) or the P/sandpaper covered MV3 (■) measuring body.

In Fig. 2A the shear rate ($\dot{\gamma}$) is plotted against shear stress (σ) for low σ for a homogenized tomato suspension of 30% tomato paste and 32.7 °Brix. The measurements were carried out with a constant stress Deer PDR Rheometer. The minimum stress which could be applied was ca 3 N/m^2. $\dot{\gamma}$ was determined after ca 3 min. At a stress of ca 40 N/m^2 the shear rate increases suddenly by a few orders of magnitude. This point was defined as the yield stress.

During overshoot experiments at low constant shear rate (0.024 s^{-1}) the shear stress firstly rises to a maximum value at a shear strain of about 0.5, followed by a decrease. This maximum shear stress corresponds well with the yield stress as determined according to the method as described above (Table 2). An example of an overshoot measurement is given in Fig. 2B.

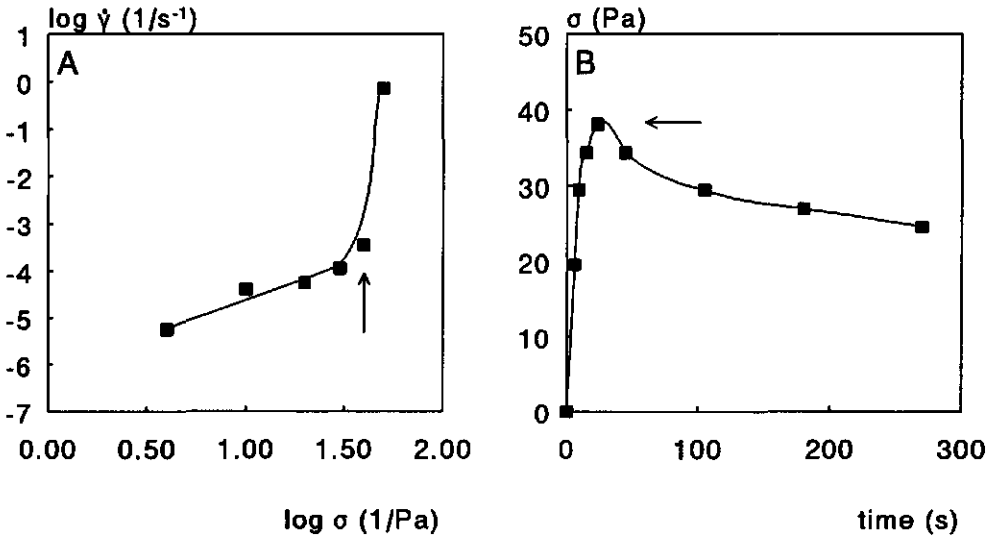

Figure 2. Determination of yield stress for a tomato suspension (30% hot break paste), homogenized at 17 MPa. **A.** Shear rate ($\dot{\gamma}$) as a function of shear stress (σ); **B.** Shear stress (σ) as a function of time at a shear rate ($\dot{\gamma}$) of 0.024 s^{-1}. Arrow; stress which is taken as an estimate of the yield stress.

Table 2. Yield stresses of two samples as determined by constant stress measurements (Deer) and by overshoot measurements (Haake).

Sample	Yield stress (Pa)	
	Constant stress (Deer)	Overshoot (Haake)
A	45	42
B	27	27

The viscoelastic character of the undisturbed tomato suspension was further studied by rotational sinusoidal oscillating experiments. To avoid damage of the network structure during the experiment, measurements must be done in the so called linear region (Whorlow, 1992). As is clear from Fig. 3 where G', is plotted as a function of the maximum applied shear strain, γ, for a homogenized tomato suspension the linear region is below a strain of ca 4.10^{-3}. Subsequent measurements were done at lower strains. The dynamic moduli G', G'' and tan δ for a similar tomato suspension as a function of frequency (f) are plotted in Fig. 4. It illustrates the viscoelastic character of the tomato suspension. The

dynamic parameters including tan δ increase with increasing f. At low f tan δ has lower values indicating a more solidlike behaviour. The higher tan δ at high f is probably due to long macromolecules which only contribute to the network at higher frequencies.

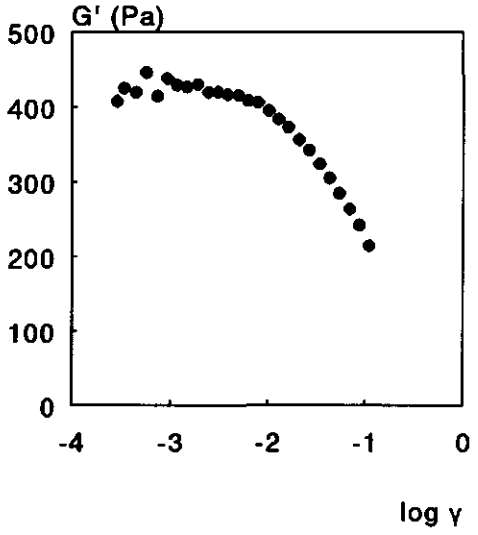

Figure 3. Strain sweep (G' as a function of strain, γ) determined at 1 Hz for a tomato suspension homogenized at 17 MPa.

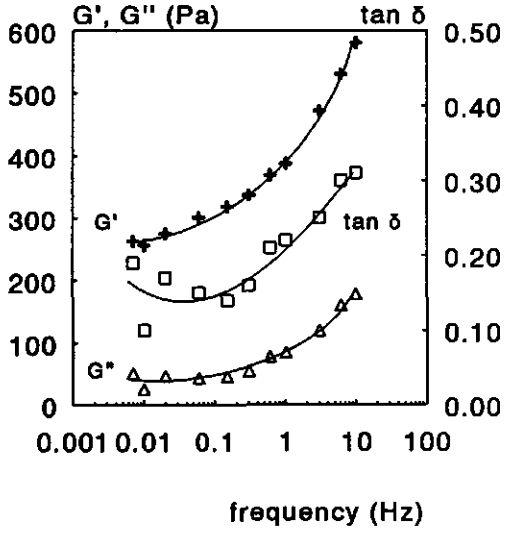

Figure 4. G', G'' and tan δ as a function of frequency (f) for a tomato suspension homogenized at 17 MPa.

2.3.2 Effect of concentration

The degree of concentration of the concentrate from which a tomato suspension is prepared, has a large effect on the apparent viscosity of cold diluted, non homogenized suspensions (Fig. 5). The apparent viscosity of the suspension prepared from the 30 °Brix concentrate is about 35% of that of the

suspension prepared from the 4.9 °Brix juice. The largest difference is between the suspensions prepared from the 4.9 and 10 °Brix concentrates. This result is in accordance with observations by Heutink (1986) who diluted concentrates between 5-30 °Brix to suspensions of 4.9 °Brix. Until now no plausible explanation has been given for this phenomenon. Heutink ascribed "dilution loss" to a complex of factors such as water removal, mechanical shear and vacuum. According to Heutink, certain ions play an important role with respect to "dilution loss". If ions had been removed before concentration by dialysis, "dilution loss" decreased significantly. Marsh et al (1977) also found that concentrating to a higher °Brix value resulted in a lower consistency of the resulting tomato suspension (12% natural tomato soluble solids, NTSS) as determined with a Bostwick viscometer. The extent of desiccation of the water insoluble fraction and the effect of it on its ability to resorb water was proposed as the reason of the observed phenomenon. A heat treatment (30 min at 100°C) after dilution of the concentrate to 12 %NTSS clearly improved consistency. It was thought that a heat treatment modifies the effect of dilution by altering the mechanism of resorption.

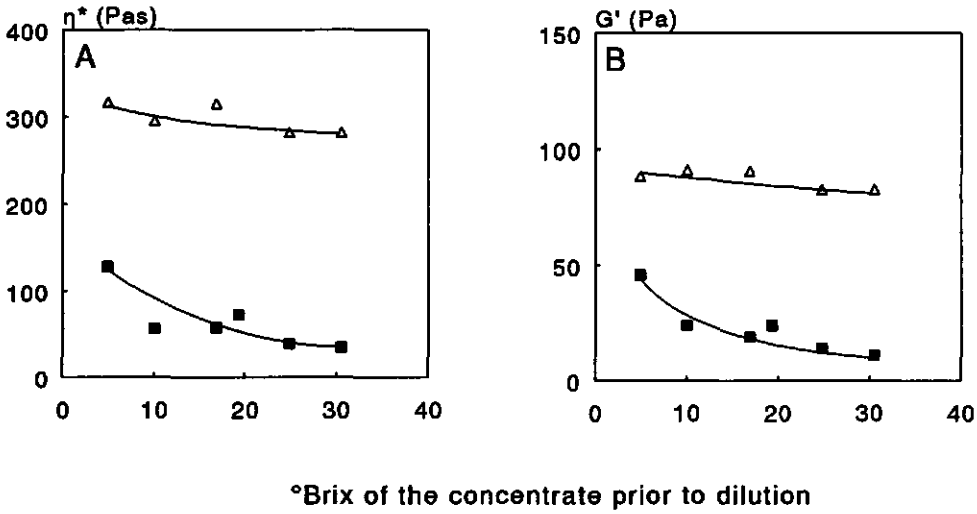

Figure 5 A. Apparent viscosity ($\dot{\gamma}$ = 0.025 s^{-1}) and **B.** G' (f = 1 Hz) as a function of °Brix of the concentrates before dilution to a suspension with 0.65% WIS, non homogenized (■) and homogenized (▵).

A possible explanation for the lower consistency of tomato suspensions prepared from a concentrate would be that chemical changes occur during the concentration process. Chemical changes in tomato cells during the concentration process of tomato juice to tomato concentrate were studied by

Heutink (1986). As a result of heating during a regular hot break tomato paste process, part of the pectin is solubilized causing a decrease in the pectin content of the WIS (Heutink, 1986). The solubilized pectin is highly esterified, which is illustrated by the decrease in degree of esterification of the unsolubilized pectin (Table 3).

Table 3. Effect of tomato processing on % pectin and % anhydrogalacturonic acid (AGA) in WIS and degree of esterification (DE) of the pectin of tomato variety H30 (from Heutink, 1986).

Product	total pectin (% of WIS)	% AGA in WIS	DE of the WIS pectin
tomato	43.1	14.6	58
hot break juice	41.3	10.9	29
hot break concentrate	40.8	9.3	28

The total quantity and the sugar composition of the hemicellulose and the cellulose fraction of the WIS did not change during the processing of the tomato to hot break paste (Heutink, 1986). In spite of some solubilization of pectin, in general the WIS/tomato solids (TS) ratio was found to remain more or less constant during the concentration process (Table 1). However, a possible role of the solubilized pectin fraction, especially the degree of esterification in the decrease of the rheological properties due to the concentration and dilution process remains unclear. So there is no clear indication that the "dilution loss" is caused by chemical changes in the cell wall.

Table 4 gives the effect of the degree of concentration of the concentrate on the particle size distribution of the diluted samples as determined by laser diffraction of the samples. Laser diffraction has been used before for particle size determination of tomato products by Getchell and Schlimme (1985) and den Ouden and van Vliet (1993). The average particle size (volume-weighted mean diameter) decreases somewhat with increasing degree of concentration. During the evaporation process used for concentrating the tomato juice, tomato cells collapse resulting in smaller cells. Heutink (1986) illustrated this microscopically and by density gradient centrifugation. Also Takada (1984) observed that most of the cells appeared to be severely folded as a consequence of concentration.

Table 4. Volume-weighted mean diameter as analyzed by a laser diffraction technique, of the different concentrates of the concentration experiment.

Concentrate (°Brix)	Volume-weighted mean diameter (μm)
4.9	391.1
10.0	381.9
16.8	369.6
19.3	370.5
24.8	370.8
30.5	360.8

Probably the decrease in apparent viscosity, due to the concentration process, can partly be explained by the smaller particles as a consequence of this process. This results in a lower volume fraction of particles at an identical WIS content. Besides a decrease in particle size also other physical changes of the cell wall could play a role in the decrease of the apparent viscosity. When a 5 °Brix tomato juice is concentrated to a 30 °Brix concentrate a volume reduction to about 16% of the original juice volume occurs. During this process the cells and cell walls are compressed which will result in deformations (e.g. bending) of the cell walls. These deformations probably lead to damaging and microscopic fracture of the rigidlike, cellulosic microfibrillar network in the cell wall, resulting in cells which are more deformable and thereby causing a lower apparent viscosity and G' of the tomato suspension.

The apparent viscosity increases due to homogenization (Fig. 5A). This has also been observed before by others (Becker et al, 1972; Hand et al, 1955; Whittenberger and Nutting 1957). The increase was relatively stronger for a tomato suspension made from a more concentrated tomato concentrate. The apparent viscosity is about 65% lower for the non homogenized tomato suspension made from a concentrate of 30 °Brix compared to the original juice, whereas after homogenization the difference is about 10-15% (Fig. 5A). The part of the decrease in apparent viscosity of the non homogenized products which can be ascribed to a smaller average particle size does not play a role in the homogenized products anymore: during the homogenization process all cells with no regard to their size are disrupted into many small particles (1-100 μm), often with fibrous appearance. Moreover, the effect of damaging and microscopic fracture of the rigidlike, cellulosic, microfibrillar network in the cell wall due to the concentration process will become much smaller because the network is completely broken down by the homogenization process.

Results of G' measured at a frequency of 1 Hz for tomato suspensions prepared from the various concentrates are presented in Fig. 5B. For the non homogenized suspensions the relative decrease of G' as a function of °Brix value of the concentrates is even stronger than the relative decrease in viscosity shown in Fig. 5A. The decrease in G' can be explained by the same factors as those which are mentioned in the case of apparent viscosity. G' increases strongly upon homogenization whereas the differences in tan δ before and after homogenization is < 0.05 (results not shown here). Tan δ had a value of about 0.3 as well for the non homogenized as the homogenized suspensions. These results indicate that, due to the homogenization process, the viscoelastic character of the tomato product does not change drastically although the shape and size of the particles does.

All non homogenized samples stored in closed, glass bottles (filling height of 10 cm) showed serum separation within one day. The layer of serum measured after 7 days for the non homogenized samples is given in Table 5. Separation of the serum is probably caused by uniaxial compression of the network under its own weight (van Vliet and Walstra, 1988). If there is no interaction between the tomato particle network and the wall of the bottle, serum separation continues until the gravitational force is counterbalanced by the product of the uniaxial compression modulus (E) of the network and the deformation gradient ϵ ($\sim \Delta h/h$, where h is the height of the network and Δh the difference in height due to the compression of the network). In formula:

$$E(\epsilon)\epsilon = \phi \Delta \rho g h \qquad (1)$$

where ϕ is the volume fraction of suspended particles, $\Delta\rho$ density difference and g acceleration due to gravity.

In order to get an impression of the validity of equation (1) a rough calculated estimate was made of Δh and the result compared with the thickness of the serum layer obtained (Table 5). An example of a rough estimate of Δh according to this equation is given here. If we assume E is about 2 times G' (taken from Fig. 5B) and $\phi\Delta\rho$ has a value of about 5 (ϕ and $\Delta\rho$ are taken as 0.05 and 100 kg/m^3, respectively) then we can estimate Δh for the suspension prepared from the 4.9 °Brix juice as 0.5 cm, which is much higher than the experimental value of 1 mm given in Table 5. For the suspensions with higher °Brix values there is better agreement between the estimated value of Δh and the measured thickness of the serum layer. Different reasons may explain discrepancies between experimental and calculated values. (i) Firstly, the values given in Table 5 are the results of the measurements of the serum layer done 1 week after the preparation of the tomato suspensions; equation (1) describes an equilibrium situation without taking into account the velocity of the network compression. The time before the equilibrium situation is reached may take more than a week.

(ii) It has been found for heterogeneous suspensions (Roefs, 1986) that the value of G' increases relatively more upon compression of the network than the value of $\phi\Delta\rho$ does. This would result in a lower Δh than the calculated one.
(iii) Another factor is (partial) adherence of the network to the wall of the bottle, which might also decrease the value of the effective h in equation (1). This in combination with the fact that only a rough estimate of $\phi\Delta\rho$ could be made, implies that no better agreement between the calculated Δh and the observed thickness of the serum layer may be anticipated.

Table 5. Amount of serum (mm) separated in bottles (height 10 cm, diameter 7 cm) from suspensions prepared from the various concentrates after 7 days and estimated values of Δh by equation (1).

Concentrate (°Brix)	Serum layer (mm)	Calculated Δh (mm)
4.9	1	5
10.0	9	8
16.8	16	14
19.3	15	17
24.8	30	21
30.5	30	25

The mechanism for serum separation represented by equation (1) also explains why the homogenized suspensions in this experiment did show much less serum separation (results not shown here). Due to a strong increase of G' as a consequence of the homogenization process, settling of the particles is strongly retarded.

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3 Particle size distribution in tomato concentrate and effect of it on rheological properties

Summary

The particle size distribution of a tomato concentrate was determined by wet sieving. Subsequently, wet sieve fractions were investigated by microscopy and a method based on laser diffraction. Both methods showed that the size of many particles is significantly larger (up to 2-3 times) than the diameter of the pores through which they have passed during wet sieving. An explanation is the deformability of the tomato cell wall. The effect of particle size distribution on rheological properties was studied by standardizing wet sieve fractions (dry matter, pH). Highest values for yield stress and apparent viscosity were found for the 90-125 and 125-180 µm wet sieve fractions.

3.1 Introduction

The particle size distribution of tomato concentrate and tomato juice has a large influence on the rheological properties (Tanglerpaibul and Rao, 1987). The particles in tomato concentrate consist mainly of separate cells or cell fragments together with some cell aggregates. These particles originate from different kinds of tissue: parenchyma, cells with round anisometric shape; vascular tissue; skin tissue containing flat rather small tightly interlocked cells covered with wax and seeds (Ilker and Szczesniak, 1990).

A technique often used for the determination of the size distribution of the particles of tomato concentrate is the wet sieving technique as described by e.g. Kimball and Kertesz (1952) and Tanglertpaibul and Rao (1987). In this technique the product is separated into fractions with different particle sizes by bringing it on top of a series of sieves of decreasing mesh size, while rinsing with an aqueous phase. It was assumed that the particles that passed through one sieve but not through the next one had an average particle diameter approximately half way between the sizes of the openings of the two sieves. For the particles which stayed on the sieve with the largest openings, the average effective particle diameter was assumed to be 50% over the size of the openings. Getchell and Schlimme (1985) analysed the particle size distribution in homogenized tomato products using a laser light scattering technique. The composite diffraction patterns obtained were converted into particle size distributions using the Fraunhofer theory. Surak et al (1979) used the Coulter Counter Model T Particle Size Analyzer for determining the particle size

distribution in tomato juice, this apparatus only measured particles between 8.0 and 161 μm.

Tanglertpaibul and Rao (1987) investigated the relation between viscosity and particle size for various tomato concentrates made from juices produced by using finisher screens with openings of 0.5, 0.7, 0.8 and 1.1 mm respectively. In general the apparent viscosity was higher for larger finisher screen openings (FSO). However, concentrates made from juice using a 0.7 mm FSO had the highest apparent viscosity. A small FSO resulted in a reduction of the particle size and in tomato concentrates with a narrow particle size distribution. Hand et al (1955) observed higher apparent viscosities for larger finisher screen openings (0.6, 0.8, 1.1, 1.5 mm). Noomhorm and Tansakul (1992) found an optimum viscosity at a screen opening of 1 mm. From literature it is clear that the finishing operation affects the consistency of tomato products, probably by influencing the particle size distribution, but also by affecting the content of water insoluble solids (WIS).

In the present study the wet sieving technique is compared with two others: (a) microscopic analysis by phase contrast microscopy; (b) a method based on analysis of the forward scattering pattern of laser light by the particles ("laser diffraction") in which composite diffraction patterns produced are converted into particle size distributions using the Fraunhofer theory.

Until now, the effect of particle size on apparent viscosity of the tomato products has been studied indirectly by changing the screen openings during the finishing process of tomato juice, resulting in products with different particle size distributions. In this study, products with different particle size distributions were obtained by wet sieving tomato paste; the resulting wet sieve fractions were standardized (i.e. on water insoluble solids, °Brix, pH) and studied rheologically (apparent viscosity, yield stress).

3.2 Experimental

Wet sieving

Wet sieving was carried out with a Fritsch sieve shaker fitted with a set of NEN 2560 sieves with pore sizes of 425, 315, 250, 180, 125, 90 and 45 μm. A 100 gram sample of tomato concentrate was diluted with water to 1 kg and then poured onto the top sieve. Subsequently the sieves were shaken for 45 min while rinsing with water (20 °C; 150 ml/min). The retained material on each sieve was determined by dry weight analysis (16-20 h at 110 °C) and expressed as a percentage of total amount of water insoluble solids (WIS).

Light Microscopy

Phase contrast microscopy was performed using a Zeiss-Axiomat microscope, magnification about 160 times. Approximately 200 particles (cells or cell aggregates) per fraction were counted and classified in particle diameter classes of 100 μm width.

Laser diffraction

Particle size distribution of diluted tomato suspensions were determined by laser diffraction using a Coulter LS 130. In this instrument laser light (wave length 720 nm) is scattered by the suspended particles and the generated diffraction pattern which is a composite of the diffraction patterns for all the particles, is measured. The instrument converts this composite diffraction pattern into particle size distributions with 72 classes ranging from 0.3 to 900 μm using Fraunhofer theory.

Preparation of tomato suspensions with varying particle sizes

An industrially prepared tomato concentrate (28 °Brix) was fractionated by wet sieving as described above. The solid material obtained consisted of water insoluble solids (WIS); soluble solids were washed away during wet sieving. The insoluble tomato solids were used for preparation of tomato suspensions with the following composition: 0.56 M NaCl, 32.2% (W/W) saccharose, 50 mM acetic acid and 1.4% water insoluble solids. The pH of the suspensions was about 3.7. Five different suspensions were prepared from respectively the wet sieve fractions: 45-90 μm; 90-125 μm; 125-180 μm; 180-250 μm and >250 μm. The ingredients were mixed to homogeneous suspensions with a Heidolph mixer and subsequently heated in 5 min to 85 °C and directly afterwards cooled to room temperature; part of the suspensions was homogenized at a pressure of 20 MPa (Rannie, Lab, MU 12:50).

Rheological measurements

Apparent viscosity measurements were performed using a Haake Rotovisco RV20, fitted with the coaxial cylinder measuring system, type P (ribbed outer cylinder) and a MV3 inner cylinder provided with sandpaper (waterproof, cornsize 80, 0.02-0.04 mm) to prevent slip. The gap width was 5 mm. Before the measurement the product was stirred several times and then poured carefully in the measuring body of the rheometer. An equilibration time of 15 minutes was taken into account in order to diminish possible thixotropic effects. The yield stress was established by doing overshoot experiments with the same apparatus. In such an experiment the shear stress is determined as a function of the time in an experiment at low shear rate (0.024 s^{-1}). The shear stress firstly

increases with increasing shear strain until a maximum after which the shear stress decreases again. The maximum shear stress was taken as the yield stress.

3.3 Results and discussion

3.3.1 Particle size distribution and comparison of methods

Table 1 shows a typical particle size distribution of an industrially prepared tomato concentrate as determined by wet sieving. The effect of the medium used for wet sieving was checked by doing the same measurement with serum from tomato concentrate instead of water. No medium effect could be observed; both serum and water gave the same results. As determined by wet sieving, the bulk of the particles has a size between 45 and 180 μm. With a maximum between 45 and 90 μm. Subsequently the size distribution of the four smallest fractions of cells were determined by phase contrast microscopy and by laser diffraction. Typical results are shown in Figure 1A and B.

Table 1. Particle size distribution as analyzed by wet sieving expressed as a percentage of water insoluble solids (WIS).

Wet sieve fraction	% of WIS
0 - 45 μm	14.3
45 - 90 μm	27.0
90 - 125 μm	23.3
125 - 180 μm	20.2
180 - 250 μm	6.6
250 - 315 μm	3.1
315 - 425 μm	3.1
>425 μm	2.4

Both the results obtained by microscopy and by laser diffraction, although not in exact agreement as such, show that the size of many particles is significantly larger (up to 2-3 times) than the notional average diameter of the pores through which they have passed during wet sieving. This result is at variance with suppositions at earlier studies (Kimball and Kertesz, 1952; Tanglertpaibul and Rao, 1987), in which it was assumed that particles which passed through one sieve but not through the next one had an average particle diameter half way between the diameters of the openings of the two sieves. An explanation for the fact that many particles are significantly larger than the openings through which they have passed may be the deformability of the tomato particles. This deformability would be expected to depend on the structure of the

original cells and cell walls.

A tomato parenchyma cell has a thin flexible wall. Especially single parenchyma cells will be deformable. From the microscopic observations it was clear that particles which had passed the smaller sieve pores (45, 90, 125, 180 μm) mostly consisted of separated round shaped anisometric cells from parenchyma tissue. Particles that were retained at the sieves with the larger sieve pores (250, 315, 425 μm) consisted mainly of aggregates of cells from skin, seeds or vascular bundles.

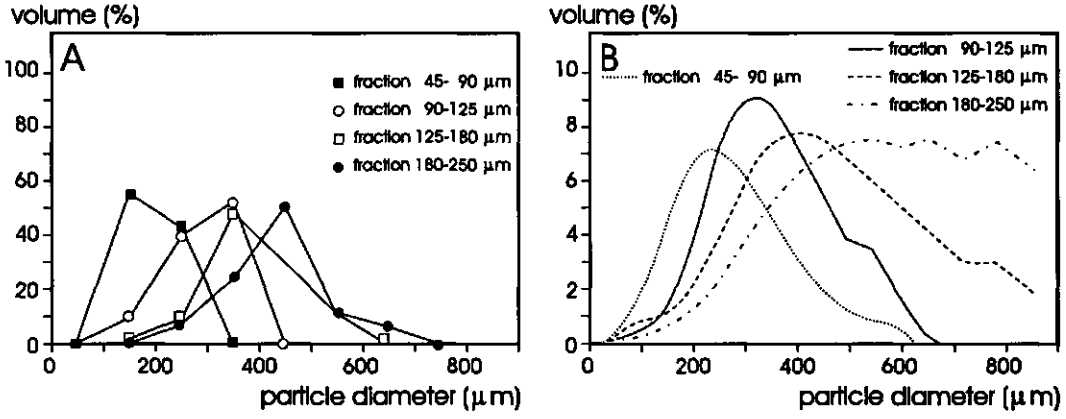

Figure 1. Approximate volume frequency distributions of different wet sieve fractions analysed by **A.** microscopy, **B.** laser diffraction

Although the flow of water is kept low there is very soon a water layer of a few mm on a sieve covered with tomato particles. This may give rise to pressures of 10-100 Pa. Moreover, pressure gradients due to shaking may exist. So during wet sieving the particles are apparently deformed in such a way that they can pass through the much smaller sieve pores.

3.3.2 Effect of particle size distribution on rheological properties

The yield stress and apparent viscosity of the tomato suspensions prepared from different wet sieve fractions are given in Fig. 2A and 2B respectively. It was found that recombination of wet sieve fractions gave rise to a viscosity increase of about 10-20% compared to the original suspension which had not been wet sieved. This increase is probably due to washing out the electrolytes during wet sieving. Whittenberger and Nutting (1957 and 1958) found that addition of NaCl and $CaCl_2$ to washed cells decreased the apparent viscosity.

A maximum in the apparent viscosity and in the yield stress is observed for

the sieve fractions 90-125 μm and 125-180 μm, both for the homogenized products and the non homogenized products. Suspensions with larger particles (>180 μm) have a significantly lower apparent viscosity and lower yield stress. At higher shear rates the dependency of η^* on average particle size was similar. From these results it can be deduced that cells from skin, seeds and vascular bundles differ in viscosity increasing effect from parenchyma cells.

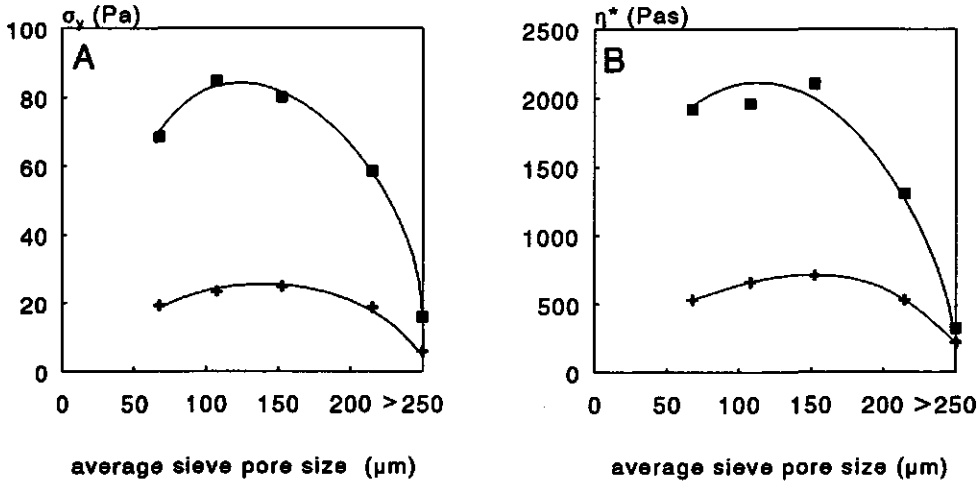

Figure 2A. Yield stress (Pa) and **B.** apparent viscosity (Pas) measured at $\dot{\gamma}=2.4 \cdot 10^{-2}\ s^{-1}$ as a function of average sieve pore size for different wet sieve fractions, WIS content = 1.4%.
Non homogenized (+); homogenized (■).

In general it can be said that the yield stress and apparent viscosity of a suspension of cell particles strongly depend on factors such as volume fraction, shape and roughness of the particles. A higher volume fraction, a stronger anisometry and more surface roughness of the particles will result in a higher apparent viscosity. In concentrated systems, which is probably the case in the experiments described here because there was no segregation after a few days, particles will be in contact with each other. Then, rheological behaviour like apparent viscosity and yield stress, depends also strongly on a property as deformability of the particles (Steeneken, 1989). As particles from sieve fraction >250 μm predominantly do not arise from parenchyma tissue, but from seeds, skin tissue and vascular bundles, they might have a higher mass density and thereby a lower volume fraction than parenchyma cells at the same weight concentration. This may be one and probably the main reason for the lower apparent viscosity and lower yield stress. Moreover, other factors mentioned above may play a role of importance.

Although a direct comparison of the results obtained in this study with those

from studies on the effect of finisher screen opening (FSO) on the apparent viscosity of juice and tomato paste is difficult, some remarks can be made here. Hand et al (1955) observed a higher apparent viscosity for products made with a larger FSO. Tangertpaibul and Rao (1987) and Noomhorn (1992) found an optimum FSO of 0.7 and 1 mm respectively at which the juice had the highest apparent viscosity. Noomhorn (1992) observed that using a screen size of 1.5 mm resulted in tomato seeds and other large particles in the juice which had a lower apparent viscosity. It must be said that the size of the particles used in this study was much smaller than the openings in the screens used during the finishing process. However, it may be expected that the average size of the tomato particles in the resulting suspensions is clearly smaller than the FSO. So the observation of an optimum FSO with respect to juice viscosity has some similarities with the outcome of this study.

As can be seen from Fig. 2A and 2B only for the smaller sieve fractions ($<250\ \mu m$) which predominantly consist of parenchyma cells, a strong increase in apparent viscosity and yield stress was observed upon homogenization. It is clear that parenchyma cells behave different upon homogenization than cells from other tissue types. This difference in behaviour of cells from different tissue types must be due to a difference in composition and structure of the cell walls.

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4 Homogenization of tomato products, apple sauce and strawberry sauce

Summary

The effect of homogenization on some physico-chemical properties of tomato suspensions, strawberry sauce and apple sauce was studied. As a result of the homogenization treatment both apparent viscosity and G' increased for tomato suspensions prepared from a hot break or a cold break paste. Tan δ decreased somewhat, indicating the network is getting a more elastic character. As a result of the homogenization treatment, the round shaped cells from tomato suspensions changed into fibrous, hairy particles. Due to the enhanced friction, these fibrous particles strongly interact with each other. Tan δ of cold break suspensions is a little lower than that of hot break suspensions homogenized at the same pressure. This is probably due to a higher cellulose content of the water insoluble solids in the cold break paste. The cell morphology of and the effect of homogenization on strawberry cells show similarities with those of tomato cells. Homogenization of apple sauce decreases its apparent viscosity. Microscopic pictures revealed that due to homogenization, apple cells are broken into regular, non fibrous particles, which slide much easier along each other than the hairy, fibrous particles in homogenized tomato suspensions.

4.1 Introduction

Homogenization of tomato products increases the apparent viscosity. This phenomenon was already recognized in 1954 by Luh et al who used a laboratory homogenizer for the homogenization of tomato paste. Increase of apparent viscosity of tomato products upon homogenization has also been observed by others (Hand et al, 1955; Whittenberger and Nutting, 1957 and 1958; Ehpraim et al, 1962). The explanation given for this viscosity increase varied. Sometimes it is ascribed to a decrease in average particle size and thereby an increase in total surface area of the suspended particles (Luh et al, 1954; Ehpraim et al, 1962), in other cases fragmentation of the predominantly spherical cellular particles into elongated particles is mentioned as cause for the increase (Hand et al, 1955; Whittenberger and Nutting, 1957 and 1958).

Viscosity of the serum was found to be unaffected by the homogenization treatment (Whittenberger and Nutting, 1957; Crandall et al, 1975).

Besides tomato products, the effect of homogenization has been studied also for some other products from plant origin. Crandall et al (1988) observed a

significant decrease in apparent viscosity of orange concentrate due to homogenization. Homogenization also decreased thixotropic behaviour of orange concentrate. The number of long filamentous fibres was reduced resulting in a product which had more smaller regular shaped particles.

In this study the effect of homogenization on the physico-chemical properties of tomato suspensions prepared from hot and cold break tomato concentrate was studied. Special attention was paid to changes in rheological properties (apparent viscosity, dynamic moduli) particle size and particle configuration.

As is obvious from literature the apparent viscosity of some products of plant origin increases upon homogenization, whereas that of others decreases. In order to get a better understanding of the reason for this difference in the effect of homogenization on apparent viscosity of fruit products and to be able to relate it to cell wall structure, strawberry and apple sauce were studied as well.

4.2 Experimental

Determination of water insoluble solids (WIS)

About 20 g of tomato product was centrifuged in a Sorval RC-5B at 30.000 g for 20 min. After decanting the supernatant, the residue was washed with water (circa 70 gram) at room temperature. This was repeated 4 times, then the supernatant had a refractive index of 0 °Brix. The residue was dried in an oven at a temperature of 110 °C (16 h), weighed and the result expressed as % WIS.

Preparation of tomato suspensions

Tomato suspensions were prepared from either an industrial cold break tomato paste or an industrial hot break tomato paste. Some analytical characteristics of these pastes are listed in Table 1.

Table 1. Composition of hot and cold break paste.

	Hot break paste	Cold break paste
Dry matter (%)	30.2	38.8
°Brix	29.5	38.3
WIS (%)	5.2	4.8
cellulose (% of WIS)	26.4	32.3
pH	4.06	4.27

The hot break paste had an extremely low pH. The tomato suspensions were standardized at 33 °Brix, pH 3.7 and 0.58 M NaCl by mixing the tomato paste with water, sugar, NaCl and acetic acid. The hot break and the cold break suspensions had a water insoluble solids content of 1.25% and 1.35% respectively. Subsequently the suspensions were heated to 90 °C and homogenized at about this temperature.

Preparation of apple sauce

Apple sauce was made from Golden Delicious apples by cutting them in steam atmosphere in small pieces (1-3 mm) which were directly heated by steam injection to 85 °C and held for 10 min at that temperature. The pieces were pressed through a finisher screen (750 rpm) with openings of 0.8 mm. The obtained sauce was subsequently cooled to room temperature. Part of the sauce was homogenized at that temperature.

Preparation of strawberry sauce

The strawberry sauce was an industrial product prepared from cold break strawberry juice. The sauce had a pH of 3.4 and a refractive index of 29 °Brix. Homogenization took place at room temperature.

Homogenization

Homogenization was done with a Rannie homogenizer, Lab model, type MU 12:50 (capacity about 100 l/h), equipped with a ribbed homogenization valve.

Light microscopy

Phase contrast and polarized light microscopy were performed using a Zeiss-Axiomat microscope with a magnification of about 160 times.

Laser Diffraction

A rough impression of the size distribution of the particles was obtained by a laser diffraction technique using a Coulter LS 130 system. In this instrument, laser light (wavelength 720 nm) is scattered by the particles, and the generated diffraction pattern, which is a composite of the individual diffraction patterns of all the particles, is measured. This composite diffraction pattern is converted into a particle size distribution with 72 classes ranging from 0.3 to 900 μm using the Fraunhofer theory.

Rheological measurements

Apparent viscosity measurements were done using a Haake Rotovisco RV20 rheometer, fitted with the coaxial cylinder measuring system, type P (ribbed

outer cylinder) and a MV3 inner cylinder provided with sandpaper (waterproof, cornsize 80, 0.02-0.04 mm) to prevent slip. The gap width was 5 mm. An equilibration time of 15 minutes was taken into account in order to diminish possible thixotropic effects. All rheological measurements were done at 20 °C. Dynamic measurements were carried out with a Bohlin VOR rheometer with a 30 mm sherated parallel plates measuring system, gap width was 2 mm. An equilibration time of 15 minutes was taken into account.

In order to determine serum viscosities, tomato suspensions were centrifuged for 20 min at 30.000 g in a Sorval RC-5B centrifuge. Subsequently the serum was filtered through Schleicher & Schüll membrane filters (5 and 1 μm). Viscosity of the serum was determined using an Ubbelohde capillary viscometer.

Neutral sugar analysis and anhydrogalacturonic acid content of serum

Both determinations took place after dialysis of the serum against 50 mM NaAc buffer pH 4.2. Total neutral sugar content of the dialyzed serum was determined by an automated orcinol-sulphuric acid method (Tollier and Robin, 1979). The anhydrogalacturonic acid content of the serum was determined colorimetrically by an automated 3-phenylphenol test (Thibault, 1979).

Cellulose content

Cellulose content was estimated quantitatively after extraction of pectin and hemicellulose from the WIS with 4 M NaOH and 0.26 M $NaBH_4$ during 8 h at room temperature (Voragen et al, 1983).

High performance size exclusion chromatography (HPSEC)

Serum was injected on a series of Biogel TSK 40, -30, -20 XL columns in series with a TSK guard column (75 x 7.5 mm). Elution was performed with 0.4 M Na-acetate buffer pH 3.0 at a flow rate of 0.8 ml/min at 30 °C. Detection was with a Shodex SE 61 refractive index detector at 40 °C.

4.3 Results

4.3.1 Homogenization of tomato suspensions prepared from hot and cold break paste

The average particle size of tomato suspensions prepared from hot break paste or cold break paste decreases with increasing homogenization pressure as follows both from laser diffraction (Table 2) and from microscopic observations (Fig. 1). In Table 2 the surface-weighted mean diameter ($d_{3,2}$) and diameter-weighted mean diameter ($d_{2,1}$) are given of hot and cold break suspensions homogenized at various pressures.

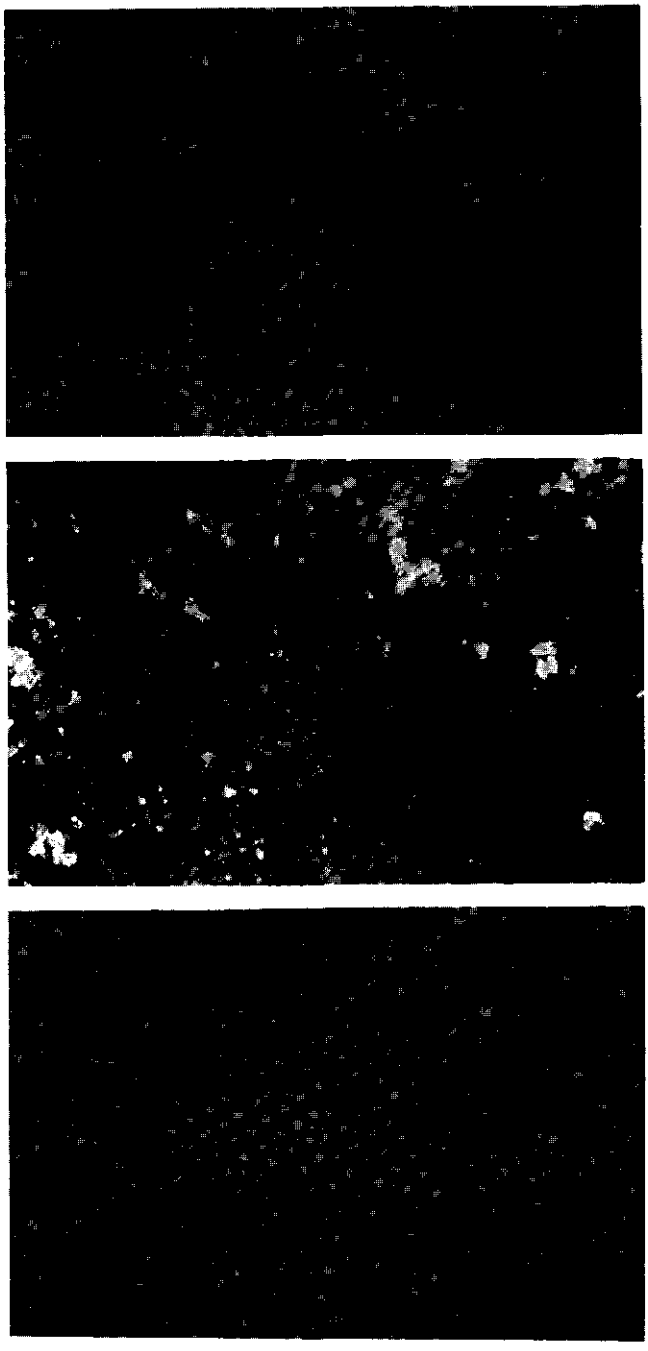

Figure 1. Microscopic pictures of tomato suspensions, 1 cm = 80 μm: **A.** hot break, non homogenized, phase contrast; **B.** hot break, non homogenized, polarized light; **C.** hot break, homogenized (20 MPa), phase contrast.

9. Extractiemethoden om het gehalte aan actief stremsel in kaas te bepalen, zijn vanwege hun lage recovery zeer aanvechtbaar.

10. De uitspraak van Umberto Eco dat creativiteit niet gelegen is in het vinden van nieuw materiaal, maar in herschikking van het bestaande, is niet geheel juist.
Umberto Eco, De volkskrant 16 januari 1995.

11. In de milieudiscussies over verpakkingsafval krijgt meermalig verpakkingsglas onevenredig veel aandacht.
Milieuplan Glas verpakkingen Branche Vereniging Glas.

12. Tomatenpuree heeft uitstekende vetvervangende eigenschappen.

13. Het moment bepaalt of de balans doorslaat.

Stellingen bij het proefschrift "Physico-chemical stability of tomato products" van F.W.C. den Ouden te verdedigen op 6 juni 1995 te Wageningen.

Stellingen

1. De mate van ordening en de fysische staat van het cellulosenetwerk in de celwanden van plantecellen is bepalend voor de viscositeitsverandering bij het homogeniseren van plantecelsuspensies.

Dit proefschrift, hoofdstuk 4.

2. Homogenisatie van tomatecelsuspensies leidt tot een toename van het elastische karakter.

Dit proefschrift, hoofdstuk 4.

3. Enzymatische hydrolyse van geconcentreerde tomatecelsuspensies met polysaccharide afbrekende enzymen bevordert serumseparatie.

Dit proefschrift, hoofdstuk 5 en 6.

4. Het "plakken" van tomatecelsuspensies aan verpakkingsmateriaal, verhoogt de stabiliteit tegen serumseparatie.

Dit proefschrift, hoofdstuk 7.

5. Microscopische breuk van het microfibrillaire cellulosenetwerk speelt een essentiële rol bij de viscositeitsverliezen die optreden door het concentreren en terugverdunnen van een tomatecelsuspensie.

Dit proefschrift, hoofdstuk 2.

6. De door Stoforos en Reid ontwikkelde methode om serumvorming in tomatenketchup te meten is eigenlijk een permeabiliteitsmeting en geen bepaling van de serumvorming.

Stoforos and Reid, 1990. J. of Food Sci. 55, 1626-1629.

7. De aanduiding "hairy region" voor het rhamnoserijke gedeelte van het pektine-molekuul zou beter vervangen kunnen worden door "branched region" of "ramified region".

Schols et al, 1990. Carbohydr. Res., 206, 117-129.

8 Niet gemodificeerd sojaeiwit is ongeschikt als basis voor biologisch afbreekbaar plastic.

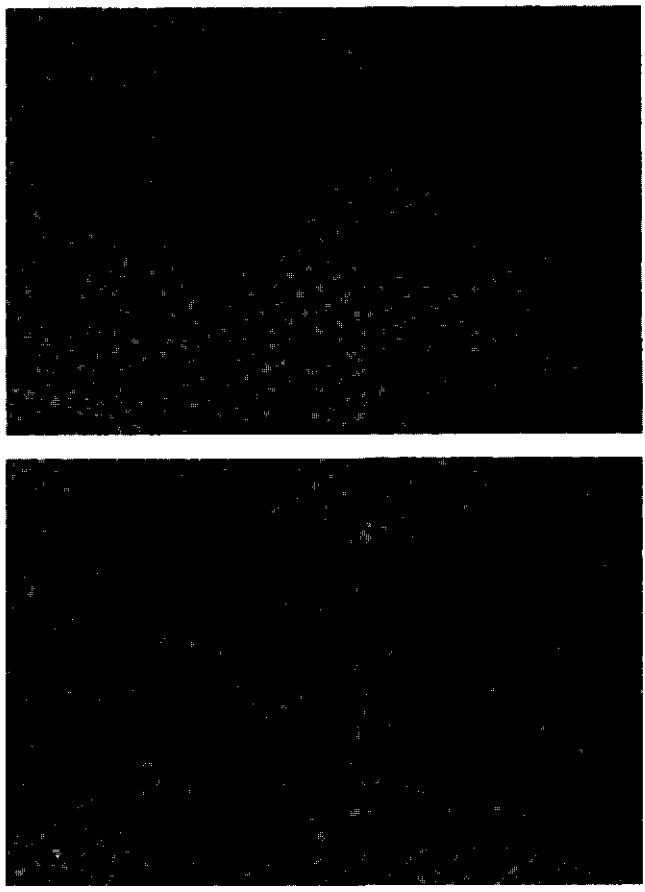

Figure 1. Microscopic pictures of tomato suspensions, 1 cm = 80 μm: **D.** cold break, non homogenized, phase contrast; **E.** cold break, homogenized (20 MPa), phase contrast.

Table 2. Surface-weighted mean diameter (d_{32}) and number-weighted mean diameter (d_{10}) as determined by laser diffraction of hot and cold break tomato suspensions as a function of homogenization pressure.

Homogenization pressure	Hot break		Cold break	
	d_{32} (μm)	d_{10} (μm)	d_{32} (μm)	d_{10} (μm)
non homogenized	121.5	0.97	50.8	0.25
10 MPa	78.7	0.83	25.3	0.22
20 MPa	47.5	0.74	14.4	0.20
30 MPa	28.6	0.30	13.2	0.20
40 MPa			11.7	0.20

Microscopic observations show that (i) in a cold break suspension the tomato cells are much more disrupted than in a hot break suspension (compare Fig. 1A and 1 D) and (ii) homogenization causes a complete disruption of the cells and cell aggregates from the suspensions of both paste types (Fig. 1). Wet sieving, which is often used for determination of particle size distribution of tomato products (Kimball and Kertesz, 1952; Tangertpaibul and Rao, 1987; den Ouden and van Vliet, 1993) can not be used to study the change in particle size with increasing homogenization pressure, because the average size of the particles homogenized at pressures above 20 MPa is smaller than the diameter of the smallest pore size of the sieves (ca 30 μm).

The hot break product homogenized at a pressure of 10 MPa still had some intact parenchyma cells as could be observed by phase contrast microscopy. Products homogenized at pressures of 20 MPa or higher contained hardly any intact parenchyma cells. All cells with exception of those from skin tissue and seeds had been disrupted. The cell fragments in homogenized tomato products have an elongated form and a fibrous appearance. By polarized light microscopy tomato cells and cell fragments only showed a slight brightening with the exception of lycopene granules which showed intense brightening (Fig. 1B). This slight brightening is caused by the rather thin cell wall and it probably indicates that cellulose fibrils in the tomato cell wall are organized in a rather disordered matter. These observations are in agreement with transmission electron micrographs presented by Becker et al (1972) from which it was clear that cellulosic microfibrils in tomato cell walls are tightly matted and interwoven in a chaotic way.

In contrast to the particles in a non homogenized hot break suspension the particles in a non homogenized cold break suspension are mainly cell fragments with fibrous appearance (Fig. 1D). The tomato cells are not intact anymore. This

must be due to hydrolysis of the pectins by the pectolytic enzymes (pectin esterase and polygalacturonase) during the cold break process combined with the forces acting on the cells during the concentration process of tomato juice to paste. The difference in particle morphology between hot and cold break tomato suspensions shows that the pectin fraction forms an essential part of the cell wall for keeping its integrity.

The effect of homogenization of tomato suspensions of hot and cold break paste on the apparent viscosity at a shear rate ($\dot{\gamma}$) of 85.7 s^{-1} is about the same (Fig. 2). In both cases apparent viscosity increases strongly upon homogenization, especially between non homogenized and 10 MPa there is a strong increase. As discussed above homogenization causes an increase in the number of fibrous particles. Probably this results in a stronger mutual friction and maybe in the formation of some entanglements between the more brushlike particles and so in a higher apparent viscosity. For homogenization pressures of around 20 MPa and higher, the increase flattened off.

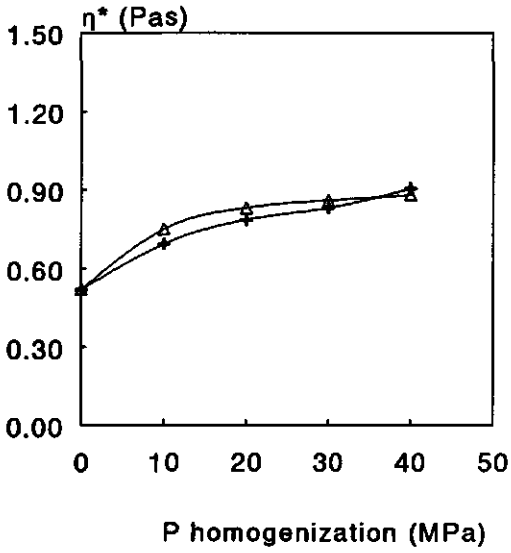

Figure 2. Apparent viscosity (η^*) measured at shear rate of 85.7 s^{-1} as a function of homogenization pressure for suspensions prepared from hot break (+) and cold break paste (△).

In Fig. 3 the shear stress is depicted as a function of shear rate for a non homogenized hot break suspension and one homogenized at 40 MPa. Measurements were done at increasing shear rates followed by decreasing shear rates. The stronger hysteresis of the curve shows that homogenization results in more pronounced thixotropic behaviour. The explanation might be that when the homogenized products start to flow, fibrous particles will on average align themselves more along the stream lines and clusters/entanglements are broken, subsequently apparent viscosity decreases.

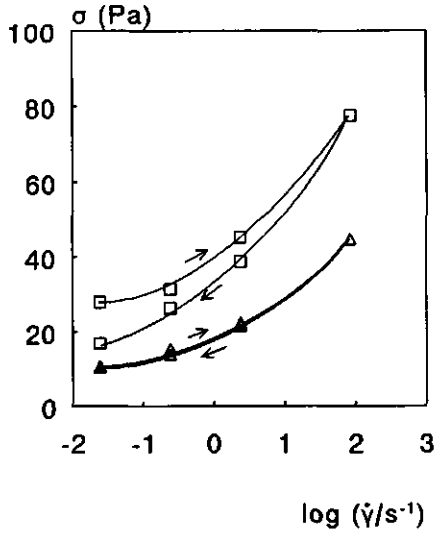

Figure 3. Shear stress as a function of shear rate for hot break suspensions; non homogenized (▵) and homogenized (40 MPa; □).

Dynamic measurements were performed at a shear strain of about $2 \cdot 10^{-3}$, which is well within the linear region. Results for G' as a function of frequency are depicted in Fig. 4 for hot break (4A) and cold break (4B) suspensions respectively, homogenized at various pressures. For both types of suspensions G' was higher when the product was homogenized at 10 MPa. Especially the cold break suspension showed a strong increase.

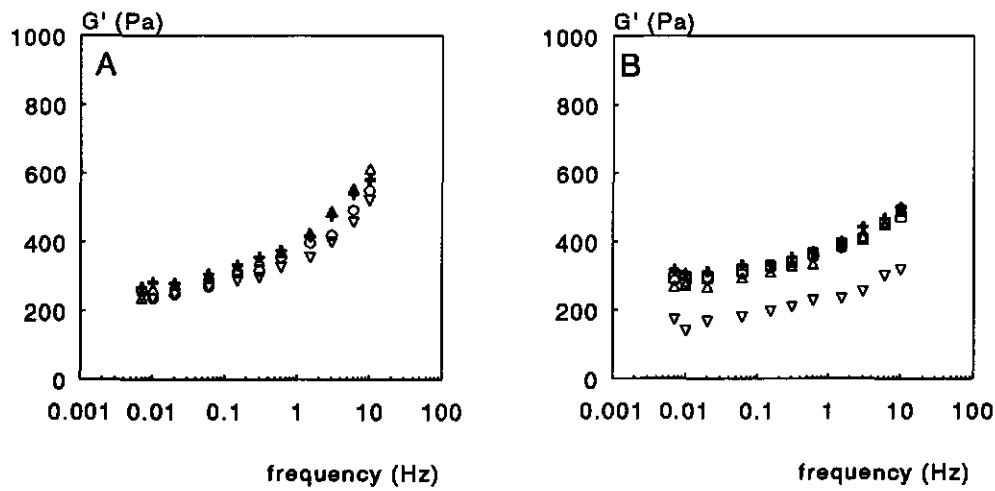

Figure 4. *G′* as a function of the frequency for suspensions prepared from **A.** hot break paste *homogenized at various pressures and* **B.** *cold break paste homogenized at various pressures;* ▿ = non homogenized, ▵ = 10 MPa, ○ = 20 MPa, + = 30 MPa, □ = 40 MPa.

For full packed systems as the non homogenized tomato suspensions G' will be proportional to the modulus (stiffness) of the particles. After homogenization the character of the system has changed completely. Particles are disrupted resulting in much smaller particles with a fibrous brushlike appearance which

probably are more or less hooked in each other. The extent of it is probably an important factor for G'. Tan δ for hot and cold break suspensions is shown in Fig. 5. For both tomato suspensions homogenization resulted in somewhat lower values of tan δ. The differences were small but reproducible. This decrease in tan δ upon homogenization means that the network had obtained a more elastic character. For all suspensions a minimum of tan δ was found around a frequency of 0.03 Hz. Probably this is related to the composite character of the network (chapter 2) but an exact explanation can not be given.

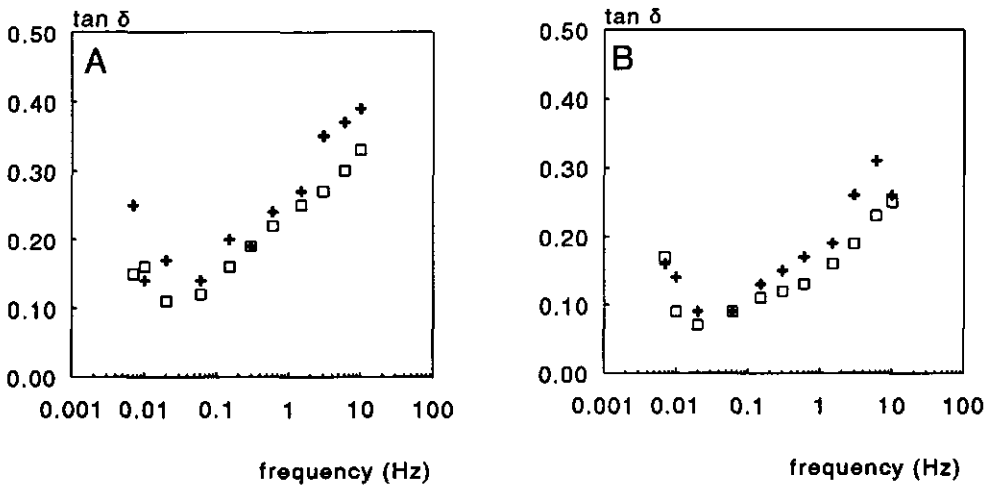

Figure 5. Tan δ as a function of the frequency for non homogenized (+) and homogenized (30 MPa; □) suspensions prepared from **A.** hot break paste and **B.** cold break paste.

Comparison of the values of tan δ for the cold break suspensions with those for the hot break suspensions homogenized at the same pressure (Fig. 5) shows that for suspensions from cold break paste tan δ is lower. This means that the cold break suspensions have a more elastic character than the hot break suspensions. This will be discussed further in the next section.

Serum viscosity as a function of homogenization pressure is shown in Fig. 6 for as well cold break as hot break tomato suspensions. Due to hydrolyzing activity of pectolytic enzymes, serum viscosity of cold break suspensions is much lower than that of hot break suspensions. Besides, it is obvious that serum viscosity of cold break suspensions does not change upon homogenization whereas that of hot break suspensions increases somewhat upon homogenization.

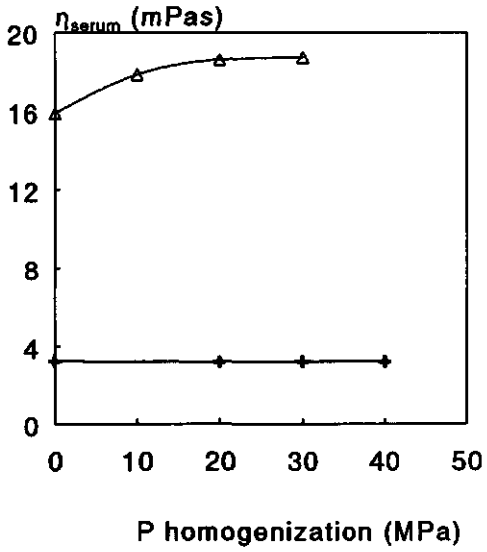

Figure 6. Serum viscosity of suspensions prepared from hot break (Δ) and cold break (+) paste.

This increase in viscosity must be due to either solubilization of macromolecules from the WIS fraction or release from this fraction of particles with a size smaller than 1 μm, which is the size of the membrane filter pores used for the preparation of the serum. The molecular weight distribution has been analyzed qualitatively by HPSEC. From Fig. 7 it is clear that the molecular weight distribution of the solubilized macromolecules of the sera of the homogenized and the non homogenized hot break suspensions are approximately the same, just as for the cold break suspensions.

The anhydrogalacturonic acid content and the neutral sugar content from the dialyzed sera (Table 3), is higher for serum from a homogenized than from a non homogenized hot break suspension. Because there is no indication that the increased serum viscosity is due to the solubilization of macromolecules (Fig. 7), the increase in serum viscosity is probably due to the release of particles of hot break suspensions with a size smaller than 1 μm, during the homogenization step. These small particles are able to pass the membrane filters used for the isolation of the serum from the tomato suspensions. Suspensions prepared from cold break paste do hardly show this behaviour upon homogenization indicating that probably the fraction of the WIS involved has already been solubilized during paste manufacture due to the action of pectolytic enzymes .

Figure 7. HPSEC patterns of serum from hot and cold break tomato suspensions before and after homogenizing at a pressure of 30 MPa.

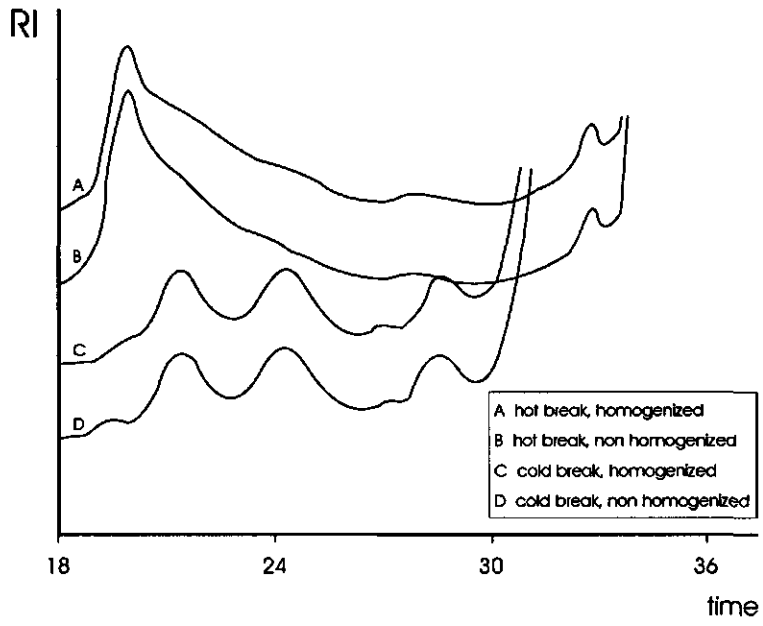

Table 3. Anhydrogalacturonic acid (AGA) and neutral sugar (NS) content in mg/ml of the serum after dialysis, from cold and hot break suspensions homogenized (30 MPa) and non homogenized.

	AGA (mg/ml)	NS (mg/ml)
HB, non homogenized	2.3	1.3
HB, homogenized	2.9	1.8
CB, non homogenized	3.2	1.6
CB, homogenized	3.4	1.6

4.3.2 Homogenization of strawberry sauce and apple sauce

Homogenization of fluid tomato products causes an increase in apparent viscosity, whereas homogenization of orange juice results in a decrease in apparent viscosity (Crandall et al, 1988). In order to understand the underlying mechanism(s) responsible for either a decrease or increase in apparent viscosity, apple sauce and strawberry sauce, have been studied too upon homogenization.

Figure 8. Microscopic pictures of apple sauce, 1 cm = 80 μm: **A.** non homogenized, phase contrast; **B.** non homogenized, polarized light; **C.** homogenized (20 MPa), phase contrast.

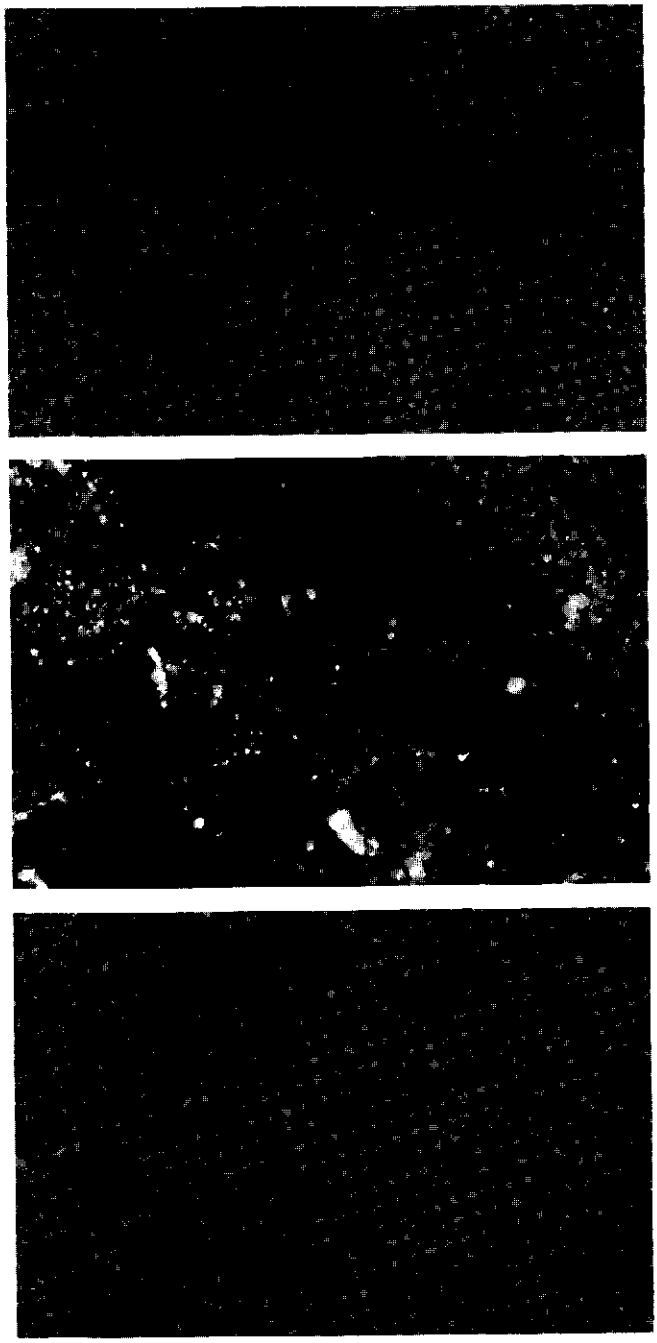

Figure 9. Microscopic pictures of strawberry sauce, 1 cm = 80 μm: **A.** non homogenized, phase contrast; **B.** non homogenized, polarized light; **C.** homogenized (20 MPa), phase contrast.

The surface-weighted mean diameter, as measured by laser diffraction both for strawberry and apple sauce products decreases upon homogenization (Table 4). This could be confirmed qualitatively by microscopic observations (Fig. 8 and 9). From those observations it is clear that the two types of fruit products have totally different cell morphologies. Particles in non homogenized apple sauce often are clusters of several cells with a size sometimes up to 700 μm. Upon homogenization at a pressure of 20 MPa, the clusters fall apart into separated cells, most cells become damaged and broken into regular pieces.

Table 4. Surface-weighted mean diameter ($d'_{3,2}$) as measured by laser diffraction (Fraunhofer) of apple sauce and strawberry sauce before and after homogenization at 20 MPa.

	apple sauce $d'_{3,2}$ (μm)	strawberry sauce $d'_{3,2}$ (μm)
non homogenized	171.9	75.3
20 MPa	65.6	9.6

Polarized light microscopy showed significant brightening of the cell walls revealing that (semi-)crystalline structures, probably mainly cellulose, are present in the relatively thick cell walls. The cells in strawberry sauce are more similar to tomato cells: almost no brightening components (with the exception of lycopene granules) could be observed in the cell walls by polarized light microscopy indicating the absence of larger (semi-)crystalline structures in the cell wall. Probably the cellulose is present in a more amorphous mode. After homogenization the cells are completely ruptured and fallen apart into long shaped filamentous cell fragments which are in shape and appearance comparable to those in homogenized tomato suspensions.

In Figure 10 the effect of homogenization on the apparent viscosity for apple sauce and strawberry sauce is shown. The apparent viscosity of apple sauce decreases upon homogenization whereas that of strawberry sauce increases. Besides, homogenized strawberry sauce is much more thixotropic than homogenized apple sauce.

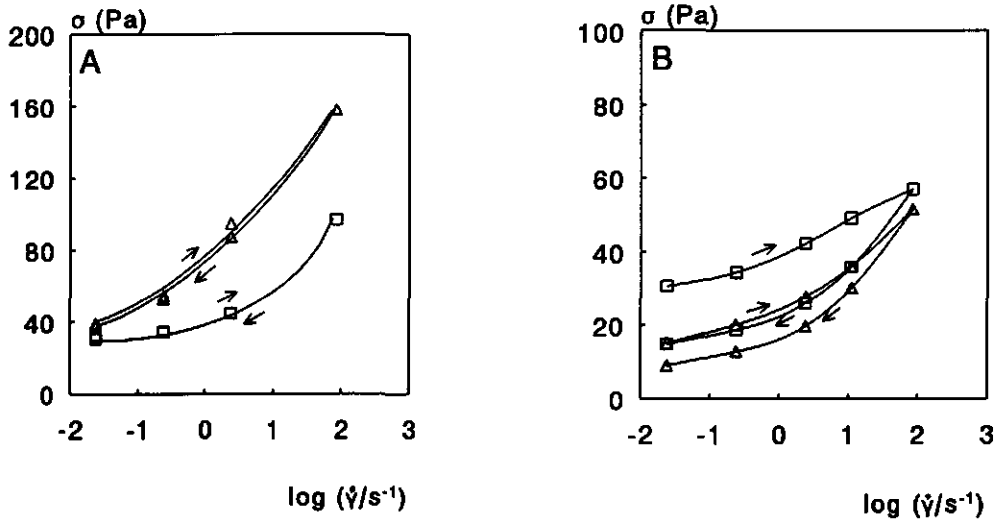

Figure 10. Shear stress as a function of shear rate for **A.** apple sauce and **B.** strawberry sauce; non homogenized (△) and homogenized (20 MPa; □).

4.4 Discussion

During homogenization of plant cell suspensions external stresses are acting on the cells and cell particles. If these external stresses exceed the fracture stress (σ_f) of the cell wall, fracture of the cell may occur. Whether fracture occurs depends on e.g. the duration of the applied stress and the deformation (strain) of the cell. Fracture mechanics of food systems is quite complicated, it has been described extensively by van Vliet et al (1991, 1993). The fracture behaviour and fracture stress of plant cells depend on a lot of factors such as origin, variety, ripeness, etc. (Vincent, 1993).

The stresses exerted on fruit cells during homogenization will depend on the flow pattern and the strain rates in the homogenizer. Depending on the Reynolds number (*Re*) flow is laminar or turbulent (Davies, 1972). *Re* is defined as:

$$Re = \rho v l/\eta \qquad (1)$$

where ρ is density of the liquid (kg/m^3), v liquid flux (m/s), l the length (m) and η viscosity of the liquid (Pas).

From the product flow and the measured width within the homogenization valve it can be calculated that v in the homogenization valve is about 10^2 m/s, η^* is

roughly estimated to be about 0.02-0.05 Pas (by extrapolating log σ versus log $\dot{\gamma}$ curves), and ρ is about 10^3 kg/m^3. Then *Re* is about $2 \cdot 10^3$, implying flow is turbulent. In turbulent flow the local flow velocity u varies in a chaotic way and the fluctuations can be characterized by u', which is the root mean-square average difference beteen u and the overall flow velocity (Walstra, 1993). If the turbulence is isotropic, the flow can be characterized according to the Kolmogorov theory. According to this theory $u'(x)$ in an energy bearing eddy, which will be mainly responsible for cell fracture, equals $C\,\epsilon^{1/3}\,x^{1/3}\,\rho^{-1/3}$, where x is eddy size, ρ is mass density and C a constant of the order of unity, ϵ is the power density, the average amount of energy dissipated per unit time and unit volume. The pressure fluctuations, responsible for fracture are in the order of $\rho\{u'(x)\}^2$ (Walstra, 1993). Cell disruption may occur if this term exceeds its fracture stress (σ_f). For a homogenization pressure of 20 MPa ϵ will be around 10^{12} W/m^3; supposed $x \approx 10^{-5}$ m, then pressure fluctuations of about $4 \cdot 10^5$ Pa will occur. Fracture of cells and parts of the cells will occur if their fracture stress is lower, except if the size of the particles is smaller than x.
As is clear from the microscopic observations (Fig. 1, 8 and 9) plant cells may fracture upon homogenization, the mode and extent depending on the species.

Some workers ascribed the increase in apparent viscosity upon homogenization to a decrease of particle size during the homogenization process and the accompanying increase in particle surface (Luh et al, 1954; Ehpraim et al, 1962). However, decrease of particle size does not automatically result in an increase of apparent viscosity as has been demonstrated for apple sauce. Whether apparent viscosity decreases or increases, strongly depends on the morphologic changes of the fruit particles during homogenization. Homogenization of plant cell suspensions results in two effects. At first, if pressure is high enough, it causes cell fracture resulting in smaller cell fragments and thereby a larger total surface area. Secondly, the physical properties e.g. shape, roughness, deformability etc. of the resulting particles may be different from the original ones. As is clear from Fig. 1, 8 and 9 depending on the type of fruit and therewith on the cell wall structure, homogenization of cells leads to cell fragments either with a more regular, roundish shape (apple) or with more elongated, fibrous shapes (tomato, strawberry). Rupture into cellular fragments with a more roundish, regular shape, similar to the original ones results in a decrease of apparent viscosity. However, rupture into cellular fragments with fibrous appearance leads to a higher apparent viscosity than that of the original cell suspensions. Also the extent of thixotropy depends on the shape of the particles. Strawberry sauce and tomato suspensions behave more thixotropic after homogenization. Apple sauce hardly exhibits any increased thixotropic behaviour upon homogenization. Besides particle shape the

extent of deformability of the particles may also have an effect. Probably the cells of the apples are stiffer and thereby less deformable. The larger stiffness of the particles is probably caused by thicker cell walls in combination with the crystalline structure of the cellulose fibrils (Newman et al, 1994).

Fracture behaviour of plant cells depends on the cell wall structure. The cellulose content of the WIS of the tomato cells is approximately 30% (Heutink, 1986), which is about at the same level as that for apples (Voragen et al, 1983). There are indications that organization and spatial distribution of the cellulose microfibrils in the cell wall play a crucial role in fracture behaviour. The organization of the cellulose in the apple cell is probably more regular (crystalline) as appeared from the polarized light microscopic photos. Our hypothesis is that this results in a relatively high fracture stress (σ_{fr}) and if homogenization pressure is high enough to induce fracture, it causes fragments with a more or less regular shape to be formed. Cellulose in tomato cells and strawberry cells is on the same scale present in a more amorphous mode. This probably results in a lower σ_{fr} and after fracture in filamentous, hairy particles which exhibit strong friction if they are sheared along each other, resulting in an increase of η^*.

Homogenization of tomato suspensions leads to products with a somewhat more elastic character, as appears from the slight decrease of tan δ (Fig. 5). Suspensions prepared from cold break paste have a lower tan δ than suspensions from hot break paste both having had the same homogenization treatment. Probably the explanation for this behaviour can also be ascribed to the role of cellulose in the cell wall. Cellulose is an unbranched polymer of D-glucopyranose residues joined by β-(1-4)-linkages. In the primary cell wall the degree of polymerisation is either ca 500 or 2500-4500 (Fry, 1988). Compared to other biopolymers in plant cells, cellulose has unique properties. Because of the stiff β-(1-4)-linkage and the absence of carbohydrate side chains, cellulose molecules form microfibrils, held together by H-bonds and Van der Waals forces. These cellulose microfibrils have a rigidlike and elastic character; they have a very high tensile stress of about 10^9 Pa (Vincent, 1993). The biological function of cellulose is presumed to be skeletal, providing shape and strength to the cell wall. It is possible that a network, consisting of cell wall polymers, containing a higher proportion of cellulose microfibrils, behaves more elastic, expressed in a lower value of tan δ. This might explain why suspensions from cold break paste have lower values for tan δ. In cold break paste, part of the pectins have been solubilized by the action of pectolytic enzymes during paste manufacture. This results in a relatively higher percentage cellulose in the WIS compared to that from hot break paste (Table 1). Besides cellulose, the cell wall consists of pectins and hemicelluloses. These polysaccharides have in common

that they often have side branches. Many of these polysaccharides consist of sugar units linked to each other by the flexible α-(1-4)-linkage (e.g. homogalacturonan). It is thought that the cellulose microfibrils are embedded in a pectin-hemicellulose matrix (Carpita and Gibeaut, 1993). Because of the flexible nature of the pectins and some polymers of the hemicellulose fraction, they probably contribute relatively more to the viscous properties of the particle network leading to a higher value of tan δ. By homogenizing a tomato suspension there is a kind of transition from a network consisting of round shaped cells to a network consisting of fibrous, hairy particles. In this fibrous network the cellulose microfibrils which have a much higher σ_{fr}, than the cell wall as a whole, might have a relatively larger contribution to the network character than in the original cell suspension. This could explain the lower value of tan δ after homogenizing (Fig 5).

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5 The effect of some polysaccharide degrading enzymes on physico-chemical properties of tomato products

Summary

Tomato suspensions from hot break paste were treated with highly purified enzymes of fungal origin: rhamnogalacturonase (RG), rhamnogalacturonan acetylesterase (RGAE), endo-arabinase (EA) and two endo-glucanase types (EG-a and EG-b). These enzymes demonstrated one or two main activities towards well specified substrates, moreover minor activities were measured. The decrease in water insoluble solids (WIS) due to the enzyme treatments after 8 h was 7.2%, 3.6%, 6.2% and 3.6% for the tomato suspensions treated with RG/RGAE, EA, EG-a and EG-b respectively. By enzyme treatment small particles are released from the tomato cells as was shown by laser diffraction particle size measurements. Values of rheological parameters decreased due to enzymatic hydrolysis. Treatment with EA and EG-b resulted in the relatively largest decrease of apparent viscosity (η^*), serum viscosity (η_{serum}), yield stress (σ_y) and storage modulus (G'). By homogenization of the enzyme treated suspensions, the rheological parameters changed drastically. Enzyme treated diluted pastes, which had lowest values for yield stress and apparent viscosity before homogenization, had highest values for these rheological parameters after homogenization. G' of all samples was about the same after the homogenization treatment. The enzyme preparations studied gave rise to more serum separation. Possible relationships between the various physico-chemical parameters on microscale (<μm) and macroscale (>μm) are discussed.

5.1 Introduction

Polysaccharide degrading enzymes like pectinases, cellulases and hemicellulases are used widely in the fruit juice industry as processing aid e.g. during maceration and liquefaction of fruits or clarification of fruit juices (Pilnik and Voragen, 1991). Degradation of cell wall polysaccharides by enzymes affects the rheological properties of processed fruits (Siliha, 1985). The technical enzyme preparations used for these applications mostly contain a mixture of active enzymes. Highly purified and well characterized polysaccharide degrading enzymes are used as a tool for elucidation of the chemical fine structure of plant cell wall polysaccharides (Voragen et al, 1993).

The water insoluble solids from tomato representing the water insoluble cell wall material consist of pectin (40-45%), hemicellulosic substances (25-30%)

and cellulose (30-35%). These cell wall components determine to a large extent the rheological and physical properties of tomato suspensions (Heutink, 1986).

In this study the effect of relatively highly purified fungal enzymes was investigated on the physical as well as the chemical properties of tomato suspensions. The enzymes studied were: rhamnogalacturonase (RG), rhamnogalacturonan acetylesterase (RGAE), endo-arabinase (EA) and two endo-glucanase types (indicated in this chapter as EG-a and EG-b). Rhamnogalacturonase hydrolyzes glycosidic linkages between rhamnosyl and galacturonopyranosyl residues of the rhamnogalacturonan backbone in the rhamnose-rich regions (Schols et al, 1990[a]). These rhamnose-rich regions are highly branched with side chains of arabinans, galactans and arabinogalactans predominantly attached to *O*-4 of the rhamnopyranosyl residues and with single unit ß-D-xylose residues linked to *O*-3 of the galacturonosyl residues. The galacturonosyl units are partially methoxylated and acetylated (Renard et al, 1993; Schols et al, 1990[b]). These parts of the pectin fraction are often indicated as "hairy" or "ramified regions" (de Vries et al, 1982; Schols et al, 1990[b]). Rhamnogalacturonan acetylesterase hydrolyzes acetyl groups from galacturonic acid units of the rhamnogalacturonan backbone in the hairy region of pectin. The enzyme acts synergistically with rhamnogalacturonase (Searle-van Leeuwen et al, 1992). Endo-arabinase shows optimal activity on linear arabinans (Rombouts et al, 1988; Voragen et al, 1987). Depending on their specific mode of action fungal endo ß-(1-4)-glucanases hydrolyze the internal ß-(1-4)-linkages of e.g. cellulose derivates, insoluble cellulose fibrils and xyloglucans.

5.2 Experimental

Characterization of enzyme preparations

The enzyme preparations were from the Department of Food Science, Food Chemistry Group of the Wageningen Agricultural University (The Netherlands). Glycanase and glycosidase activities of the enzyme preparations towards specified substrates were tested according to the Nelson-Somogyi method (Somogyi, 1952) by which the increase of reducing endgroups is measured. All enzymatic reactions were performed with substrate concentrations of 0.5% (w/v) in 50 mM sodium acetate buffer, pH 5.0 at 37 °C. The following substrates were used: polygalacturonic acid (Fluka, Switzerland); citrus pectin, degree of esterification (DE) 70% (Sanofi, France); branched arabinan from beet (British sugar, UK); linear arabinan (Megazyme, Australia); type II arabino galactan (*β*-1,3,6) (Stractan, USA); type I arabino galactan (*β*-1,4) from soy; arabino xylan from wheat (MegaZyme, Australia); xyloglucan; xylan from oat spelt (Sigma, USA); carboxymethyl cellulose (AKZO-Nobel, Netherlands);

Avicell, micro crystalline cellulose (Serva, Germany).

Glycosidase activities were measured using the *p*-nitrophenyl derivates of α-L-arabinofuranose, α/ß-D-galactopyranose, α/ß-D-xylopyranose, α/ß-D-mannopyranose and α/ß-D-glucopyranose as substrates (Sigma, USA). Enzyme activities towards these substrates were calculated from the release of *p*-nitrophenol from the corresponding glycoside measured at 405 nm after raising the pH to 9.0. One unit of activity is defined as the amount of enzyme that catalyses the release of 1 μmol of reducing sugar or *p*-nitrophenol per minute.

Rhamnogalacturonase activity was assayed qualitatively by high-performance size-exclusion chromatography (HPSEC). For this purpose modified hairy regions (MHR) isolated from enzymatically liquefied apples and saponified modified hairy regions (s-MHR) were incubated with enzyme preparations (1 and 24 h) in 50 mM sodium acetate buffer, pH 4.2, at 30 °C (Schols et al, 1990[b]). Digests of the enzymatic degradation were analysed on a set of BioGel TSK 40, 30, 20XL columns in series (BioRad), with 0.4 M sodium acetate (pH 3.0) as eluent, at a flow rate of 0.8 ml min^{-1}/30 °C.

Pectin esterase (PE) activity was determined with 0.5% brown ribbon pectin as substrate in solution, containing 0.15 M NaCl, at pH 4.2 and 37 °C. Released carboxylgroups were titrated with NaOH.

The protein content of the crude enzyme preparations was determined according to Sedmak and Grossberg (1977).

Determination of water insoluble solids (WIS)

Circa 20 g of tomato product was centrifuged in a Sorval RC-5B at 30.000 *g* for 20 min. After decanting the supernatant, the residue was washed with water (circa 70 gram) at room temperature. This was repeated 4 times, then the supernatant had a refractive index of about 0 °Brix. The residue was dried in an oven at a temperature of 110 °C (16 h), weighed and the result expressed as %WIS.

Determination of sugar composition

Neutral sugar compositions of water insoluble solids (WIS) were determined as alditol acetates by GLC. Hydrolysis and derivation were performed according to Englyst and Cummings (1984). Alditol acetates were separated on a 3 m x 2 mm glass column (packed with Chrom WAW 80-1000 mesh, coated with 3% OV275) in a Carlo Erba GC operated at 200 °C and equipped with a FID detector.

Total neutral sugar content was determined by an automated orcinol-sulphuric acid method (Tollier and Robin, 1979).

Anhydrogalacturonic acids (AGA) were estimated quantitatively by the auto-

mated colorimetric 3-phenylphenol method (Thibault, 1979).

Degree of esterification (DE) of pectins was determined according to the HPLC method of Voragen et al (1986). DE was calculated as molar ratio of the content of methanol and anhydrogalacturonic acid.

Enzymatic modification of diluted tomato paste

Hot break tomato paste (28 °Brix) was diluted with water to 10 °Brix and heated to about 80 °C while mixing. After cooling to 37 °C, 0.5, or 1 g/kg suspension of enzyme preparation was added and the suspensions were incubated at that temperature. For analysis of the chemical composition, the reaction was stopped after 8 h by heating to 100 °C for about 10 min.

Homogenization of tomato suspensions

Incubated tomato suspensions were mixed with saccharose, NaCl, acetic acid to pH 3.6, 32.5 °Brix, 0.56 M NaCl and 1.4% WIS. The mixture was subsequently heated to 85 °C and homogenized with a Rannie homogenizer, Lab model, type MU 12:50 (capacity about 100 l/h), equipped with a ribbed homogenization valve. After homogenizing the product was cooled to room temperature.

Rheological measurements

Apparent viscosities were measured using a Haake Rotovisco Rheometer RV20 equipped with a coaxial cylinder measuring system, type P (profiled outer cylinder) and MV3 inner cylinder covered with sandpaper (cornsize 80, 0.02-0.04 mm). All rheological measurements were performed at 25 °C.

Yield stresses were determined in overshoot measurements with the same apparatus. The shear stress was determined as a function of time at a shear rate of 0.024 s^{-1}. The maximum shear stress (σ) was taken as the yield stress (σ_y).

Dynamic measurements were done with a Bohlin VOR rheometer, fitted with sherated plates (diameter 3 cm). The gap size between the plates was 2 mm and the applied strain $2 \cdot 10^{-3}$.

Serum viscosities of tomato suspensions were measured with an Ubbelohde capillary viscometer. Beforehand, samples were centrifuged for 20 min at 30.000 g in a Sorval RC-5B centrifuge. Supernatants were filtered through Schleicher & Schüll membrane filters (5 and 1 μm).

Particle size measurements

Phase contrast microscopy, magnification about 160 times (Zeiss, Axiomat) was used in order to get a visual impression of the enzyme treated diluted paste.

A rough estimate of the particle size distribution was obtained by a method based on laser diffraction (Coulter LS 130). With this system diffraction patterns are converted into particle size distribution using the Fraunhofer theory.

Estimation of serum separation

Serum quantities formed by separation were measured by filling polypropylene bottles (diameter 6 cm) with about 350 g of tomato suspension. After 7 days storage at room temperature (at dark) the amount of formed serum was measured by decanting.

5.3 Results

5.3.1 Characterization of enzyme preparations

Enzyme activities of the various enzyme preparations towards well specified substrates are listed in Table 1. All enzyme preparations showed main activities towards one or two of the substrates. Moreover, minor activities were measured. In comparison with industrial preparations these enzyme preparations must be considered as quite pure (Pilnik and Voragen, 1991). RG showed extremely low activities towards substrates others than MHR or s-MHR. The enzyme preparations with the exception of RG showed either some activity on polygalacturonic acid or some activity on citrus pectin (70% DE). EG-b and to a far lesser extent RG exhibited PE activity (18 and 0.049 μmol/min respectively). EG-a (endo ß-(1-4)-glucanase) preferentially hydrolyzes the ß-(1-4)-linkage of xyloglucan, whereas EG-b is mainly active on soluble CM-cellulose, the specific activities of these enzymes resembles those of Endo IV and Endo I respectively as described by Vincken et al (1994). The presence of both types of enzymes in tomato fruit has been demonstrated by Maclachlan and Brady (1992). Xyloglucan is a polysaccharide which occurs in the primary cell wall of dicotyls like tomato. Its cellulose backbone which is about 0.15-1.5 μm long consists of 300-3000 ß-(1-4)-linked D-glucopyranose residues (Fry, 1989). About 60-75% of the glucose residues have side chains attached to position 6. Main side chains are single unit xylose residues. Besides other sugars can be attached e.g. galactose and fucose. There is some regularity in the distribution of these side chains. It is thought that xyloglucans form a tight monolayer on the surface of the cellulose fibrils by means of hydrogen bonds (Valent and Albersheim, 1974). Strong acid or alkali (24% KOH) is required to dissolve xyloglucan from cell wall

preparations (Hayashi et al, 1984). Xyloglucan is involved in control of cell growth, it effects cell wall extensibility and therefore the rate of cell expansion. The average chain length of xyloglucan is many times the diameter of cellulose fibrils, indicating cross links are formed by binding to adjacent fibrils and thereby contributing to wall rigidity (Carpita and Gibeaut, 1993; Hayashi and Maclachlan, 1984; McCann and Roberts, 1991).

Table 1. Specific glycanase and glycosidase activities (μmol/min/g) towards different substrates, and of the various enzyme preparations. PE = pectin esterase activity, - = not detectable, + = little activity, + + = moderate activity, + + + = large activity. MHR = modified hairy regions, MHR-S = saponified modified hairy regions. Protein content of the preparations expressed in g/g.

	RG	RGAE	EA	EG-a	EG-b
Polygalacturonic acid	0.0002	0.8	2.3	1.4	5.7
Citrus pectin	0.0006	2.5	1.7	3.1	0.00
MHR	+	-	+ +	-	-
MHR-S	+ + +	-	+ +	-	-
Branched arabinan	0.003	2.1	160	9.8	2.3
Linear arabinan	0.0	0.7	8600	2.7	9.4
Arabino galactan 1,3,6	0.0001	0.1	0.1	0.1	0.006
Arabino galactan 1,4	0.008	15	9.6	42.0	1.2
Arabino xylan	0.0002	5.6	4.3	7.8	0.6
Xyloglucan	0.0044	4.2	3.3	35000	140
Xylan	0.0004	1.4	7.0	13	0.9
CMC-Cellulose	0.0016	2.2	4.1	20	800
Avicell	0.0006	0.7	0.4	1.1	5.9
PE	0.049	-	-	-	18
α-L-ara-f	-	-	-	<1	-
α-D-gal-p	-	1	0.7	3	0.2
β-D-gal-p	1	2	1.4	13	0.6
α-D-xyl-p	-	-	-	<1	-
β-D-xyl-p	-	-	-	<1	-
α-D-man-p	-	-	-	<1	-
β-D-man-p	-	-	-	<1	-
α-D-glc-p	1	9	7	17	-
β-D-glc-p	7	122	26	95	-
Protein content	0.07	0.34	0.26	0.09	0.02

5.3.2 Effect of enzyme treatment on chemical composition

The quantity of water insoluble solids (WIS) together with the sugar composition of the WIS fractions of the enzyme treated diluted paste (0.5 g/kg) after 8h of incubation at a temperature of 37 °C is given in Table 2.

It can be seen that there is a decrease in WIS which is between 3.6 and 7.2%. The largest decrease was found for the diluted paste which had been incubated with RG/RGAE. It was also found that, as could be expected from the specific activity, a large proportion of the rhamnosyl units (about 50% of the amount present in the WIS) had gone into solution.

Table 2. Amount of WIS and high molecular weight water soluble solids (HMW-WSS) obtained by dialysis of the serum, from the various enzyme treated pastes and the sugar composition of these fractions expressed in % (W/W) from WIS and HMW-WSS respectively. AGA = anhydrogalacturonic acid, DE = degree of esterification.

	Control	RG/RGAE	EA	EG-a	EG-b
WIS	1.95	1.81	1.88	1.83	1.88
Glucose	31.3	30.7	29.8	28.3	30.9
AGA	13.0	12.0	12.5	12.7	12.4
Mannose	3.5	3.3	3.3	3.5	3.0
Xylose	3.5	3.3	3.3	3.5	3.0
Galactose	2.4	1.7	1.8	1.5	2.0
Arabinose	1.2	0.7	0.7	0.6	0.8
Rhamnose	0.4	0.2	0.3	0.3	0.4
DE	48	35	48	44	26
HMW-WSS	0.64	0.64	0.67	0.65	0.62
Glucose	3.4	2.8	3.4	2.8	2.5
AGA	40.9	40.8	41.6	41.6	41.9
Mannose	1.8	2.3	1.9	2.7	1.9
Xylose	1.0	1.1	1.1	1.1	0.9
Galactose	3.8	3.9	3.8	3.8	4.1
Arabinose	2.5	2.7	2.5	2.5	2.8
Rhamnose	1.1	1.1	1.1	1.3	1.1

For all enzyme treated pastes there is a significant decrease of arabinose and galactose. These sugar units, including that of rhamnose, are typical sugars from the "hairy regions" of pectin (Renard et al, 1993). The ratio anhydrogalacturonic acid:neutral sugars solubilized by RG/RGAE is about 2.6:1, which is significantly higher than that from earlier studies on RG (Renard et al, 1993). However it must be said that these studies were conducted on apple in

stead of tomato. The EG-b and RG/RGAE treated paste had a clearly lower degree of esterification, which can be expected from the presence of some pectin esterase activity (Table 1).

The quantity and sugar composition of high molecular weight-water soluble solids (HMW-WSS) measured after dialysis of the serum is also given in Table 2. The main portion of high molecular weight soluble solids of all sera consisted of anhydrogalacturonic acid. For all samples the total amount of HMW-WSS and sugar composition was about the same. This probably points to the fact that the hydrolyzed water insoluble solids had been degraded to small molecules with a polymerisation degree below about 5 which are able to pass the dialysis tube.

5.3.3 Effect of enzymes on rheological properties and particle size distribution

In Fig. 1 apparent viscosity (η^*) and yield stress (σ_y) are given as a function of incubation time. Both rheological parameters decreased as a function of time (enzyme dosage: 0.5 g/kg diluted paste).

Diluted paste incubated with endo-arabinase preparation shows the strongest decrease. This can be due either to the activity of the enzyme preparation towards arabinans or to contaminating, hydrolyzing activity towards pectin and polygalacturonic acid (Table 1). Because of the impurity of the enzyme preparation it cannot be stated which enzyme to what extent is responsible for the decrease in apparent viscosity and yield stress. This can be said in general for all enzyme preparations. Although relatively pure in composition, compared to commercial preparations (Pilnik and Voragen, 1991) they all demonstrate some side activities. Especially polygalacturonase may have a large effect on the apparent viscosity (Heutink, 1986). Changes found in physical properties cannot be ascribed to the hydrolyzing activity of one enzyme only.

The arabinase tested was a fungal endo-arabinase, it hydrolyzes glycosidic linkages in linear parts of arabinan backbones. Endo-arabinase from *Aspergillus* shows optimal activity on linear arabinan with progressively decreasing activity as the linear sequences are more substituted (Voragen et al, 1987). The enzyme acts synergistically with other arabinan degrading enzymes (arabinosidase I and II). About 2.1 to 4.6% (W/W) of the polysaccharides from the WIS fraction consists of arabinosyl units (Heutink, 1986). Approximately half of these arabinosyl units is present in the hemicellulose fraction, the other half is present in the pectin fraction. Linear arabinans are probably absent in the hemicellulosic fraction of tomato cell walls . However, they are found in the branched, ramified parts of the pectin fraction (Heutink, 1986). From this it can be deduced that the endo-arabinase tested, predominantly hydrolyzes arabinans present in the "hairy regions" of the pectin fraction of tomato WIS.

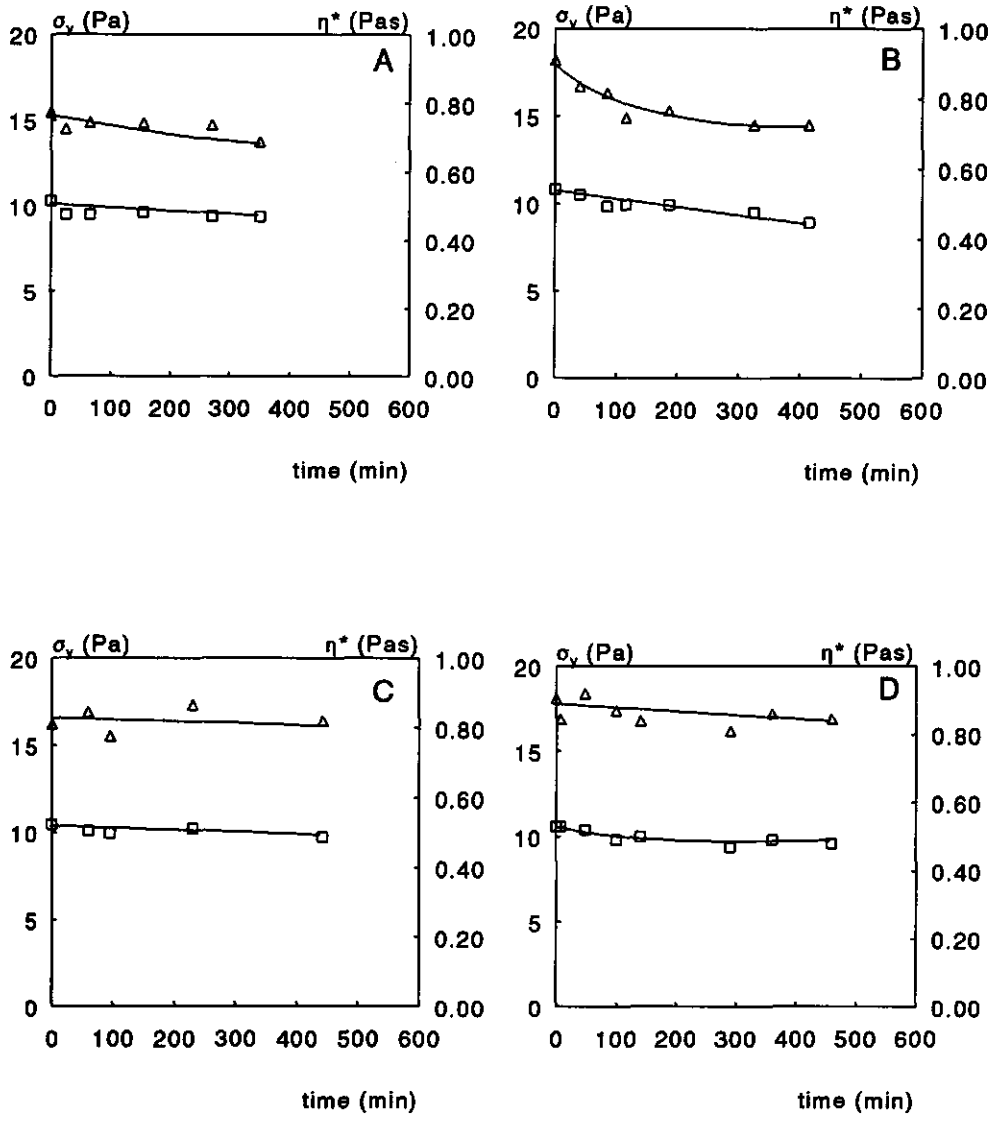

Figure 1. Yield stress (σ_y; ▵) and apparent viscosity (η^*; □) determined at a shear rate of 85.7 s^{-1} of the various enzyme treated tomato suspensions as a function of incubation time (enzyme dosage 0.5 g/kg tomato suspension). **A**: RG/RGAE, **B**: EA, **C**: EG-a, **D**: EG-b.

G′ and tan δ are depicted as a function of incubation time in Fig. 2, the enzyme dosage was 0.5 g/kg tomato suspension. In general values of G' fluctuate more than those of η^* and σ_y. G' shows about the same trend as η^*

and σ_y, but less pronounced (Fig. 2), whereas tan δ remains more or less unchanged; the viscoelastic character does not alter in spite of the hydrolyzing activities of the enzymes.

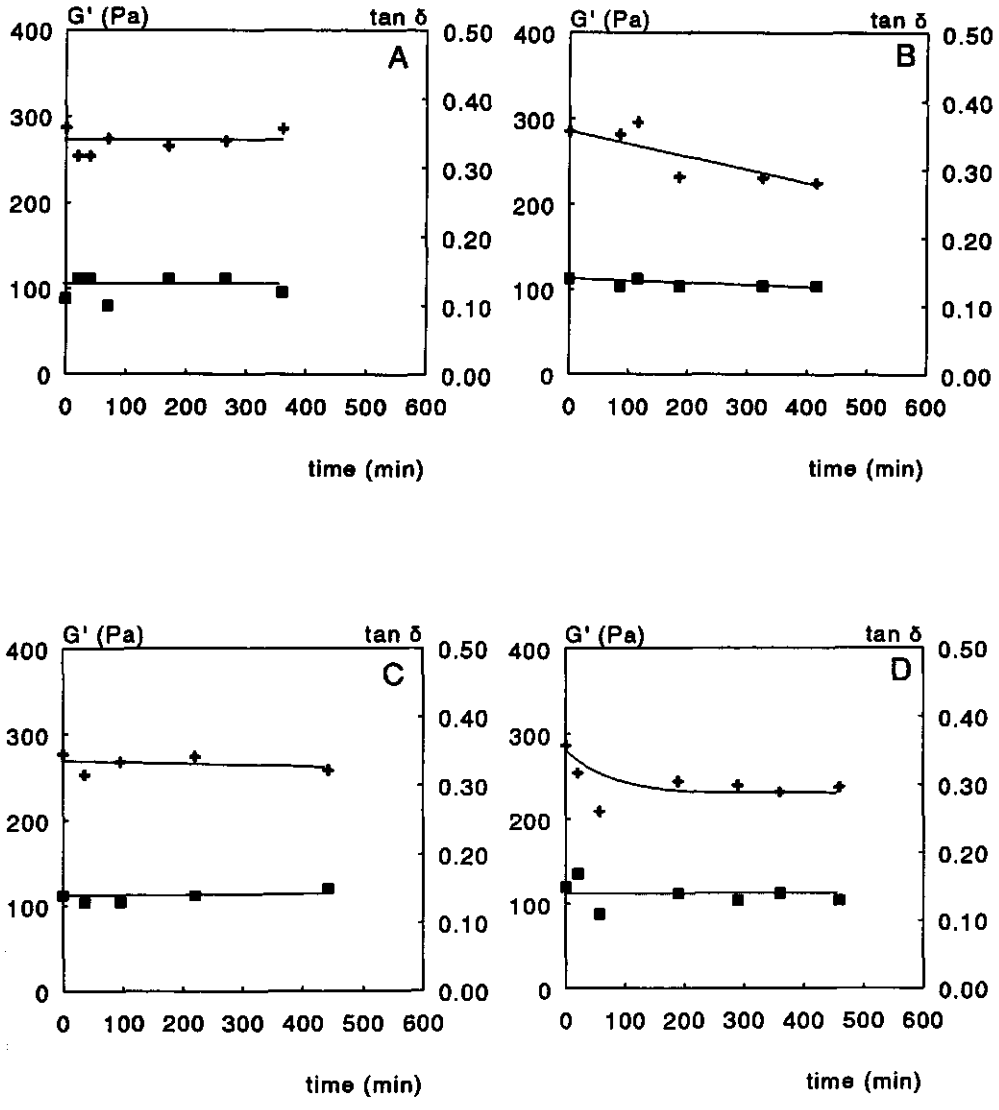

Figure 2. G' (+) and tan δ (■) of the various enzyme treated tomato suspensions as a function of incubation time (enzyme dosage 0.5 g/kg suspension). **A:** RG/RGAE, **B:** EA, **C:** EG-a, **D:** EG-b.

Serum viscosities of EG-a, EG-b and EA treated diluted paste decrease drastically (Fig. 3) by the hydrolyzing activity of the enzymes. The trend is not in exact agreement with that for the apparent viscosity and yield stress. EG-b demonstrates the largest effect on serum viscosity. This enzyme preparation shows special activity towards carboxymethyl-cellulose and to a lesser extent towards xyloglucan. Glucans are the main portion of the insoluble cell wall material of the tomato; about 60% of the insoluble polysaccharides consists of glucosyl units. Largest part of this is present in the cellulose fraction. EG-a as well as EG-b show only slight activity towards Avicell (Table 1) which is an insoluble, crystalline cellulose. As cellulose in tomato cell walls is insoluble too, it indicates the cellulose fraction will probably remain more or less intact after EG (a and b) incubation. A possible explanation for the strong decrease of the serum viscosity due to EG-b would be the relatively high activity of this enzyme preparation towards polygalacturonic acid (Table 1).

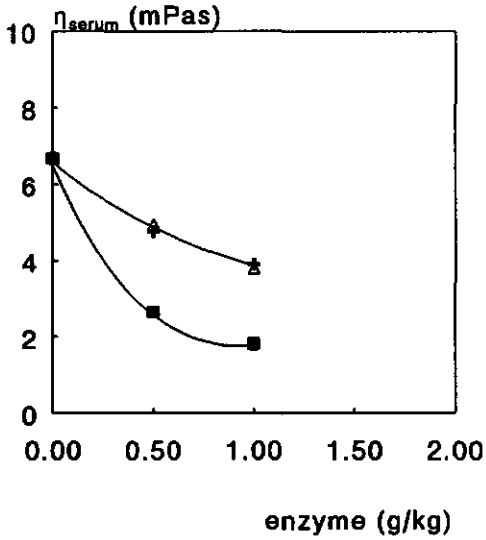

Figure 3. Serum viscosity as a function of enzyme dosage after 8 h of incubation. EA:▲, EG-a: +, EG-b: ■.

In general the decrease of serum viscosity as consequence of the enzymatic degradation is more pronounced than the decrease of apparent viscosity of the whole suspension. Soluble polysaccharides have a good accessibility for the enzymes and thereby are a much better substrate than the insoluble polysaccharides from the WIS fraction. From Table 2 it is clear that the sera of all enzymatically modified pastes have about the same concentration of water soluble high molecular polysaccharides. However, these data give no information about the polymerization degree of the solubilized macromolecules. Serum viscosity is a good indicator for the average polymerization degree of the soluble polysaccharides because it is related to the molecular weight of the polysaccharides.

5.3.4 Homogenization of enzyme treated tomato suspensions

Tomato suspensions were incubated with enzyme preparations (0.5 g/kg) during 8 h at 37 °C. After the enzyme incubation part of the product was homogenized as desribed previously. Yield stress and apparent viscosity of the homogenized as well as the non homogenized suspensions are listed in Table 3.

Table 3. Yield stress (σ_y) and apparent viscosity (η^*) determined at $\dot{\gamma}$ = 85.7 s^{-1} of the various enzyme treated tomato suspensions, non homogenized and homogenzized.

Enzyme treatment	Non homogenized		Homogenized	
	σ_y (Pa)	η^* (Pas)	σ_y (Pa)	η^* (Pas)
Control	11.4	0.46	35.6	0.78
RG/RGAE	10.1	0.42	35.8	0.79
EA	9.1	0.38	43.2	0.83
EG-a	9.1	0.41	36.3	0.83
EG-b	8.3	0.37	41.7	0.83

Enzyme treatment leads in all cases to a lower yield stress and apparent viscosity for the non homogenized suspensions. After homogenization the effect is opposite; apparent viscosity and yield stress are higher for the enzyme treated, homogenized suspensions than for the non enzyme treated, homogenized suspensions. The smallest effect is seen for RG/RGAE.

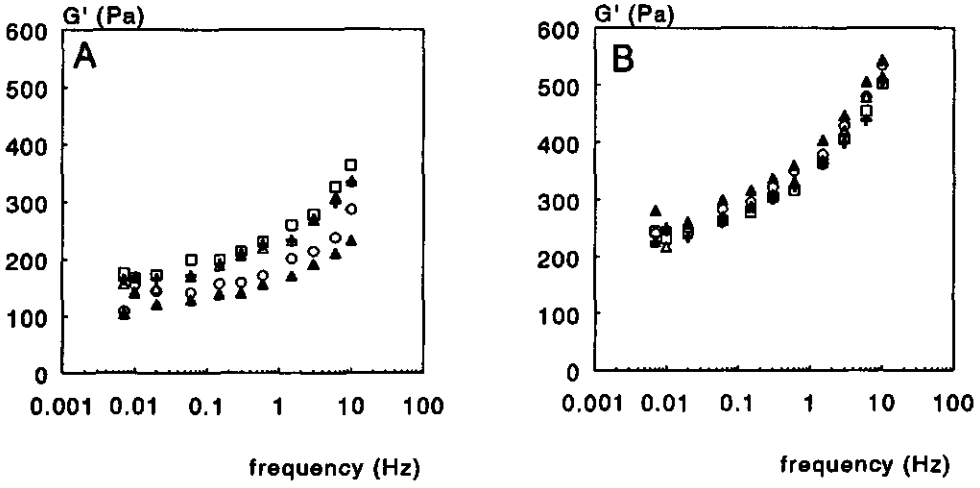

Figure 4. *G'* as a function of frequency of enzyme treated tomato suspensions, **A.** non-homogenized and **B.** homogenized (after the incubation). non enzyme treated: □, RG/RGAE: +, EA: ○, EG-a; △, EG-b: ▲.

G' versus frequency of the homogenized and the non-homogenized suspensions is depicted in Fig. 4A and 4B. In particular G' of EA and EG-b treated paste decreased by the enzymatic degradation, which is in agreement with results shown in Fig. 2. G' increases due to the homogenization, which confirms earlier found results (chapter 3). By the homogenization treatment, G' of all suspensions got approximately the same value (Fig. 4B).

Particle size of both the non homogenized and the homogenized product decreased due to the enzyme treatment (Table 4). There is no direct quantitative relationship between particle size before and after the homogenization.

Table 4. Surface-weighted mean diameter (d_{32}) and number-weighted mean diameter (d_{10}) as measured by laser diffraction of the various enzyme treated tomato suspensions, non homogenized and homogenized.

Enzyme treatment	Non homogenized		Homogenized	
	d_{32} (μm)	d_{10} (μm)	d_{32} (μm)	d_{10} (μm)
Control	125.0	1.17	31.9	0.54
RG/RGAE	114.7	0.99	32.1	0.47
EA	120.8	1.02	29.1	0.42
EG-a	117.1	1.00	29.6	0.42
EG-b	112.6	1.00	25.3	0.41

Serum separation numbers are listed in Table 5. Values are averages of 5 measurements, standard deviation numbers varied between 2 and 3 g. It can be concluded that enzyme treatments of tomato suspensions results in larger serum quantities.

Table 5. Serum quantity (g) of homogenized tomato suspensions, measured after 7 days by decanting.

Enzyme treatment	Average serum (g)
Control	7.5
RG/RGAE	9.8
EA	10.9
EG-a	10.0
EG-b	11.6

5.4 Discussion

The enzymes studied here, all affect the chemical composition and the physical properties of the tomato suspensions. In this research it has been shown that enzymatic degradation of fluid tomato suspensions has two distinct effects on microscale. At first the quantity of water insoluble solids decreased (Table 2), besides the composition of the WIS changed. However, WIS decreased only slightly, but by the solubilization of polysaccharides from the cell walls, the cell disintegrated to some extent, as follows from the particle size distribution measurements (Table 3) but also from microscopic observations. By these processes properties on macroscale e.g. rheological properties and serum separation are affected.

Changes in rheological properties like apparent viscosity and G' are not directly related to changes in WIS content and/or particle size distribution. For example treatment with RG/RGAE resulted in the largest decrease of WIS and average particle size compared to treatments with other enzyme preparations. However, properties on macroscale (rheological properties, serum separation) are affected to a much lesser extent than by the other enzymes. Besides the WIS content and the particle size also other physico-chemical characteristics are probably important with respect to the rheological properties. Some of these are: shape, deformability and higher roughness of the particles. More anisotropic shape of the particles, stiffer particles and more roughness all increase the apparent viscosity. Although an approximate impression can be obtained of the shape and roughness of the particles by using microscopic techniques, it will still be very difficult to get a quantitative idea about these parameters.

Tomato suspensions incubated with either EA or EG (a or b) showed the most pronounced changes in rheological properties (Table 3, Fig. 1, 2 and 4). Non homogenized suspensions treated with these enzymes were lowest in apparent viscosity, yield stress and G'. By homogenization, these enzyme treated suspensions got higher apparent viscosity and yield stress compared to the non enzyme treated, homogenized tomato suspension. Under these experimental circumstances, it looks like if, there is an inverse relationship between yield stress and apparent viscosity before homogenization and yield stress and apparent viscosity after homogenization.

During homogenization under high pressure, tomato cells and particles are forced to flow through the very narrow gap of the homogenization valve which has a width of a few hundreds of μm. During this process cells are torn apart to long shaped, filamentous, hairy, brushlike particles in which the hairs may be hooked in each other, resulting in large friction between the particles and so causing a higher apparent viscosity. The mechanism responsible for fracture of

the cells depends on the type of flow in the homogenization valve. As has been calculated before (chapter 4) the flow in the homogenization valve is turbulent. In that case the efficiency of the homogenizing process will depend on the energy density of the eddies. During emulsification by turbulent flow, the viscosity of the continuous phase hardly affects the resulting particle size distribution of an emulsion (Walstra, 1993). Using the same theory for a suspension of cell particles it means that the lower (serum) viscosity of the enzyme treated diluted pastes can not explain the higher apparent viscosity of these tomato suspensions after homogenization.

From fracture mechanics it is known that fracture occurs near defects or inhomogeneities (Luyten, 1988). A tomato suspension is a suspension of cells, cell aggregates and cell fragments. The cell wall, making up these particles, can be considered as a composite gel, consisting of a cellulosic, a hemicellulosic and a pectin fraction. It is plausible that some polysaccharide degrading enzymes are causing notches and weak spots in the cell wall. Because of the damaging of the cell wall, the cells and cell fragments become more deformable, thereby causing a lower apparent viscosity. During homogenizing, the cells are torn up in many particles. Maybe the weak spots in the cell wall makes the cell easier to rupture, which might lead to more fibrous particles, and thereby a higher apparent viscosity after the homogenization treatment.

As can be seen in Table 5 enzyme treated tomato suspensions especially EG-b and EA treated paste exhibit more serum separation. This phenomenon cannot be explained by the compression theory only (chapter 2 and 7). According to this theory separation of serum is caused by uniaxial compression of the network under its own weight (van Vliet and Walstra, 1988). Under ideal circumstances the network will be compressed by the gravitational force until the latter one is counterbalanced by the product of the uniaxial compression modulus of the network, which is proportional to G', and the deformation gradient (chapter 2 and 7). This implies that the serum layer would be inversely proportional to G' in a dynamic experiment. From Figure 4 it is clear that all homogenized tomato suspensions, irrespectively of their enzyme treatment, represent the same trend of G' versus frequency. With other words, a tomato suspension with relatively little serum separation does not exhibit higher G' versus frequency (Fig. 4). This indicates that other factors play a role in the serum separation process. One possible factor is local segregation of the network (see chapter 7), which will result in the formation of little channels in the suspension. Indeed, this could be confirmed by microscopic observations. Channels were seen with diameters of ten to hundreds micrometers, through which serum could flow easily and so speed up serum formation. Local segregation might be enhanced by a more pronounced disintegration of the cell

wall by some polysaccharide degrading enzymes. Moreover, serum viscosity may determine the amount of serum measured after 1 week. The velocity of serum separation is inversely proportional to the serum viscosity (chapter 7). The amount of serum after a very long waiting time will be independent of serum viscosity.

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6 Pectin esterases: their effect on physico-chemical properties of tomato products

Summary

Diluted hot break tomato paste was subjected to demethylation by pectin esterase (pectin methyl esterase; PE) from oranges or fungi. Both pectin esterase types caused an increase in the yield stress. However, the block wise deesterification induced by orange PE resulted in a much stronger increase than the random deesterification by fungal PE. This increased yield stress is thought to result from the formation of a calcium pectinate network. Apparent viscosity (86 s^{-1}) was found to be only slightly higher after treatment with orange PE or fungal PE. The calcium pectinate network is destroyed to a large extent by shearing. During PE action, serum viscosity increased initially by circa 25% to a maximum, after which it decreased to approximately 50% of the original value. This decrease is probably caused by precipitation of the calcium pectinate onto the tomato particles. This could be confirmed by the increased water insoluble solids (WIS) content after PE incubation. Homogenized orange PE treated tomato suspensions had lower apparent viscosities, yield stresses and values for G' than non enzyme treated homogenized tomato suspensions. PE incubated homogenized tomato suspensions exhibited more serum separation.

6.1 Introduction

When pectin is deesterified to low methoxyl (LM) pectin, with a degree of esterification < 45%, gelation may occur. Whether a gel is formed depends on among others the number of consecutive free carboxyl groups and the calcium activity. It is generally accepted that LM pectin gelation relies on the so called egg box model (Grant et al., 1973). According to this model junction zones are created in which galacturonans are associated parallelly through electrostatic and ionic bonding of carboxyl groups with Ca^{2+} ions.

Pectins can be deesterified either chemically at high and low pH or enzymatically by pectin esterase (PE). Other names for pectin esterase are pectin methylesterase, pectin demethoxylase and pectin methoxylase (Versteeg, 1979). Pectin esterase is synthesized by higher plants (e.g. tomato, orange) and by microorganisms. Plant PE deesterifies pectin linearly along the pectin chain, creating blocks of free carboxyl groups resulting in a very calcium sensitive form of pectin (Kohn et al, 1983; Markovic et al, 1981). It is believed that the enzyme starts near reducing ends of highly esterified pectin molecules (Lee and

Macmillan, 1970) and on the methylester groups next to free carboxyl groups (Kohn et al, 1968, 1983). In tomatoes four forms of pectin esterase have been demonstrated by chromatography (Pressey and Avants, 1972); in oranges eight forms have been identified (Versteeg, 1979). Complete inactivation of tomato pectin esterase takes place at a temperature of 80 °C during 15 sec (Garces and Luh, 1972). Cell wall solubilization by polygalacturonase is stimulated by pectin esterase (Pressey and Avants, 1982).

Fungal pectin esterase releases the ester groups randomly, in the same mode as do the alkali hydroxides (Kohn et al, 1983). Fungal PE is less resistant to heat than plant PE and has an isoelectric point (pI) and pH optimum in the acidic range (Baron et al, 1980), in contrast to the alkaline pH range (7-8) for plant PE (Axelos and Thibault, 1991).

In this research the effect of pectin esterase from oranges and fungi on the physico-chemical properties of diluted hot break tomato paste was studied.

6.2 Experimental

Orange pectin esterase

This enzyme was isolated from orange peels by the method described by Krop (1974).

Fungal pectin esterase

Fungal pectin esterase was a preparation from Gist Brocades (The Netherlands); Rapidase CPE.

Characterization of enzyme preparations

Pectin esterase activity was determined at 20 °C on 0.5% green ribbon pectin, dissolved in 0.15 M NaCl; 0.05-0.1 ml of the enzyme preparation was added and released carboxyl groups were titrated with 0.01 M NaOH with a pH-stat.

Polygalacturonase activity was assayed according to the Nelson Somogyi method (Somogyi, 1952). Trace amounts of polygalacturonase not detectable by the Nelson Somogyi test were determined by adding 20 μl of the enzyme solution to 1 ml of a 2 mg/ml polygalacturonic acid solution in 0.05 M ammonium acetate buffer, pH 4.2. The reaction mixture was incubated for 48 h at 30°C and subsequently analysed by high performance size exclusion chromatography. The elution patterns of the samples were compared with that of a control without enzyme.

Water insoluble solids (WIS)
About 20 g of tomato product was centrifuged for 20 min at 30.000 *g* in a Sorval RC-5B centrifuge. The pellet was extracted with water (circa 70 g) and centrifuged again. This procedure was repeated 4 times, then the °Brix value of the supernatant was about zero. After the last extraction the residue was dried at 110°C (16 h).

Alcohol insoluble solids (AIS)
Two volumes of 96% propanol were added to one volume of tomato product. The suspension was held for about one hour at room temperature and subsequently filtered on a Büchner funnel. The procedure was repeated twice with 60-70% propanol and once with 96% propanol. After the last extraction the residue was air dried.

Galacturonic acid
This was estimated colorimetrically by an automated 3-phenylphenol test (Thibault, 1979).

Degree of esterification (DE)
The DE of the pectin was determined according to the method of Voragen et al (1986). DE was calculated as molar ratio from the content of methanol and galacturonic acid.

High performance size exclusion chromatography (HPSEC)
Enzymatically degraded fractions of polygalacturonic acid or AIS were injected on Biogel TSK-40, -30, -20XL columns (300 x 7.5 mm, BioRad Labs, USA), in sequence. The columns were operated at 30 °C and a flow rate of 0.8 ml/min, with 0.4 M sodium acetate buffer pH 3.0 as eluent. Elution products were detected with a Shodex SE 61 refractive index detector at 40 °C.

High performance ion exchange chromatography (HPIEC)
HPIEC of AIS subfractions was performed with a Dionex BioLC system (Dionex, USA) equipped with a Dionex Carbopac PA-1 column (250 x 4 mm). The column was pre-equilibrated with 0.1 M NaOH and after sample injection eluted with two linear gradients of NaAc in 0.1 M NaOH (0.35-0.7 M, 35 min and 0.7-1 M, 5 min), washed for 5 min with 1 M NaAc in 0.1 M NaOH and then reequilibrated for 15 min with 0.1 M NaOH. The flow rate was 1 ml/min. Detection was made with a PAD II pulsed-amperometric detector (Dionex) equipped with a gold working-electrode and an Ag/AgCl reference electrode.

The pulse potentials and durations were: E_1 = 0.1 V, t_1 = 500 ms; E_2 = 0.6 V, t_2 = 100 ms; E_3 = -0.6 V, t_3 = 100 ms.

Enzymatic modification of diluted tomato paste

Hot break tomato paste (28 °Brix, 5.27% WIS) was diluted with water to 10°Brix, heated to about 85 °C while mixing. After cooling to incubation temperature the enzyme preparation was added and the suspensions were held at that temperature for various time intervals. The reaction was stopped by heating to 100 °C for about 10 min after which the chemical composition and serum viscosity were determined.

Enzymatic degradation of AIS by polygalacturonase

Endopolygalacturonase from *Kluyveromyces fragilis,* purified according to Versteeg (1979) was used to degrade AIS from non treated, fungal PE treated and orange PE treated diluted paste. 30 mg of AIS was dispersed in 2 ml 0.05 M NH_3-acetate buffer at pH 4.2 and incubated with 0.06 U of endo-PG at 30°C for 48 h.

Homogenization of tomato suspensions

Tomato suspensions were incubated with orange PE as described above. After stopping the reaction, the suspensions were mixed with saccharose, NaCl, acetic acid to obtain a suspension with pH 3.6, 32.5 °Brix, 0.56 M NaCl and 1.4% WIS. The suspensions were heated to 85 °C while mixing and homogenized with a Rannie homogenizer, Lab model, type MU 12:50, equipped with a ribbed homogenization valve. After homogenizing the product was cooled to room temperature.

Rheological measurements

Apparent viscosities were measured using a Haake Rotovisco Rheometer RV20 equipped with a coaxial cylinder measuring system, type P (profiled outer cylinder) and MV3 inner cylinder covered with sandpaper (cornsize 80, 0.02-0.04 mm). All measurements were done at a temperature of 25 °C.

Yield stresses were determined by overshoot measurements with the same apparatus. After incubation the suspension was poured gently in the measuring body. After a waiting time of 15 min, the shear stress was determined as a function of time at a shear rate of 0.024 s^{-1}. The maximum shear stress was taken as the yield stress (σ_y).

Dynamic measurements were done with a Bohlin VOR rheometer, fitted with sherated plates (diameter 3 cm). The gap size between the plates was 2 mm and the applied strain $2 \cdot 10^{-3}$ (-).

Serum viscosities of the tomato suspensions were measured with an Ubbelohde capillary viscometer. Beforehand, samples were centrifuged for 20 min at 30.000 *g* in a Sorval RC-5B centrifuge. The supernatant was filtered through Schleicher & Schüll membrane filters (5 and 1μm).

Determining serum separation

Serum quantities formed on top of the product by separation were measured by filling polypropylene bottles (diameter 6 cm) with about 350 g of tomato suspension. After 7 days the amount of serum formed was measured by decanting.

6.3 Results

6.3.1 Characterization of the enzyme preparations

The activity of the fungal PE preparation (Rapidase CPE) was about 100 U/ml at pH 4.5, which is the optimum pH. The optimum temperature of this fungal PE is 30-40 °C. After dialysis and freeze drying orange PE had an activity of 985 U/ml at pH 7.5. At pH 4.5, which is more close to the pH of tomato juice and paste, the activity was about 220 U/ml. It is known that cations activate plant pectin esterases and shift their pH optima to lower pH values. The optimum temperature of plant pectin esterases is appoximately 55 °C (Versteeg, 1979). As is seen from the HPSEC chromatogram in Fig. 1 orange PE is free of polygalacturonase whereas Rapidase CPE exhibited some PG activity, indicated by the peak at retention time 31.4 min. The HPSEC method used here for analyzing PG activity is very sensitive. No PG activity could be shown by the Nelson Somogyi test.

6.3.2 Chemical characterization of PE treated diluted pastes

The WIS content of the orange PE treated diluted tomato paste increased from 1.71% to 1.95% after 23 h (Table 1). An increase in WIS due to PE hydrolysis was also found by Heutink (1986). This increase is a result of the formation of a calcium pectinate network formed after the deesterification of the pectin by orange PE. Calcium pectinate probably precipitates on the cells resulting in a higher determined WIS content. The WIS content of the fungal PE treated paste decreased somewhat from 1.84 to 1.78%, after 23 h.

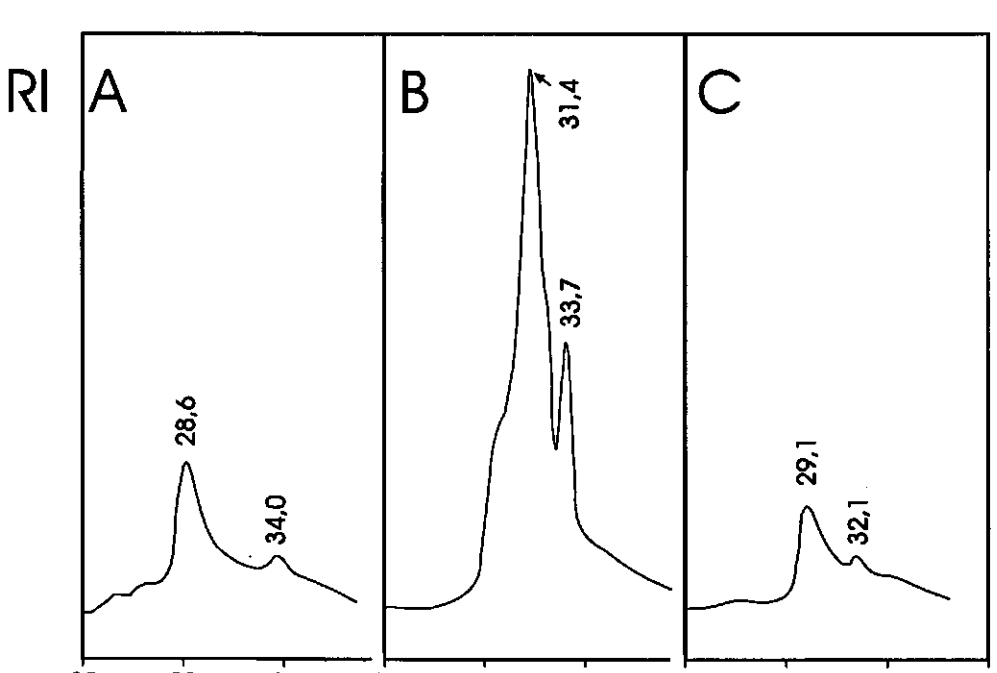

Figure 1. Elution patterns (HPSEC) of polygalacturonic acid in ammonium acetate buffer (0.05 M) after incubation **A.** control, without enzyme; **B.** incubated with fungal PE (Rapidase CPE); **C.** incubated with orange PE.

In case of the fungal PE preparation two reactions are playing a role. In the short term the random deesterification by fungal pectin esterase is probably the most important one. However, in the long term, PG, present at a very low level, is playing a prominent role, and probably responsible for the decrease in WIS content.

Table 1. WIS content of orange PE treated paste after different incubation times.

Incubation time (h)	0	2	4	7	23
WIS (%)	1.71	1.71	1.73	1.81	1.95

The galacturonic acid content of the AIS remained more or less constant (22%), this was also the case for the orange PE treated paste. The decrease in degree of esterification of the fungal PE treated diluted paste is given in Table 2. The degree of esterification of the starting material was about 40% which is quite low compared to values of around 50% found by other workers (Anger and Dongowski, 1985; Heutink, 1986).

Table 2. Degree of esterification of AIS from fungal pectin esterase treated diluted paste after different incubation times.

Incubation time (h)	0	3	7	26	47
DE (%)	38.8	14.5	10.5	6.6	6.4

The degree of esterification strongly depends on the break temperature and ripening stage of the tomatoes (Bhasin and Bains, 1987; Heutink, 1986). It appears like fungal PE is not able to deesterify tomato pectin to a degree lower than about 6%. Orange PE deesterifies until about 10% (Solms and Deuel, 1955). Deesterification catalyzed by pectin esterase from tomato stops at a DE of around 2% (Markovic et al, 1981). Plant pectin esterases are inhibited by competitive inhibitors, e.g. by pectate, one of their own reaction products (Termote, 1977).

AIS subfractions of the various diluted pastes (control and PE treated) after degradation by endo PG in acetate buffer were characterized by HPSEC and HPIEC. Also non PG treated AIS subfractions were characterized by these chromatographic methods (Fig. 2 and Table 3 respectively).

Table 3. Estimated percentages by HPIEC of pectin fractions with DP 1-5 or 6-10 which had been solubilized in an acetate buffer (0.05 M, pH 5.2). Samples were incubated with or without PG from *K. fragilis*.

Treatment	Degree of Polymerisation	AIS-pectin non treated paste (%)	AIS-pectin fungal PE treated paste (%)	AIS-pectin orange PE treated paste (%)
Control	1-5	0.2	0.6	0.2
	6-10	0.1	0.1	0.1
PG treated	1-5	2.6	4.6	3.3
	6-10	0.1	0.1	0.3

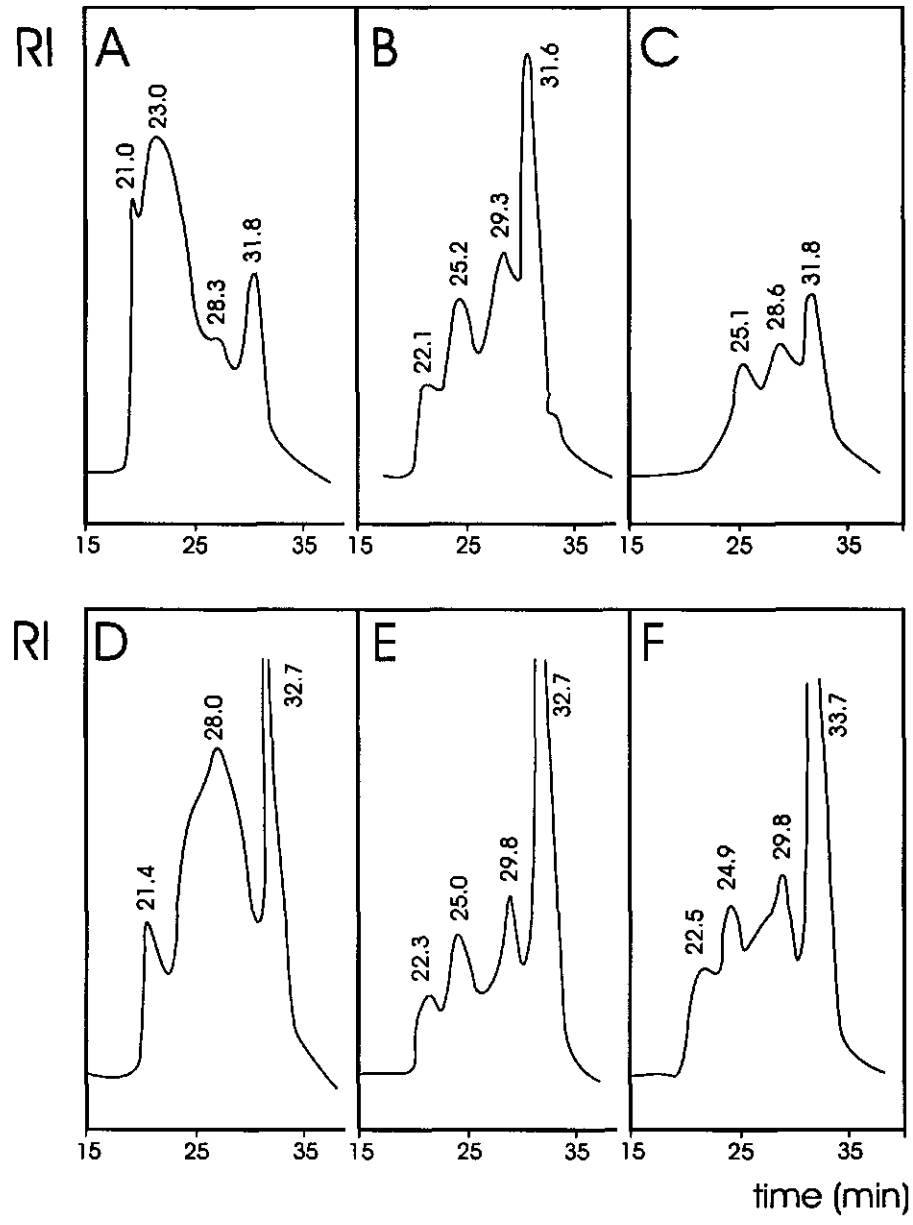

Figure 2. Elution patterns (HPSEC) of in acetate buffer (0.05 M), pH 4.2 solubilized AIS subfractions :**A.** AIS non treated paste, **B.** AIS fungal PE treated paste, **C.** AIS orange PE treated paste, **D.** AIS non treated paste after incubation with PG, **E.** AIS fungal PE treated paste after incubation with PG, **F.** AIS orange PE treated paste after incubation with PG.

Comparison of the HPSEC chromatograms of solubilized subfractions of the non PG treated AIS (Fig. 2A, B and C) shows that the subfraction from the orange PE treated AIS (Fig. 2C) contains a relatively small quantity of high molecular weight molecules with a retention time of 21-23 min compared with that from the non treated paste (Fig. 2A), which shows a large peak at this retention time. The low response of molecules with this retention time is probably a result of the blockwise deesterification of orange PE causing water soluble pectins to precipitate and so remain insoluble. Also the chromatogram from the AIS subfraction of the fungal PE treated paste (Fig. 2B) shows smaller peaks at a retention time of 21-23 min than that from the AIS subfraction of the non treated paste (Fig. 2A).

Comparison of the chromatograms of the solubilized AIS subfractions after incubation with PG (Fig. 2D, E and F) with those of the non PG incubated AIS samples (Fig. 2 A, B and C) shows that: (i) The solubilized subfraction from AIS from the fungal PE treated paste shows the smallest change as a result of PG incubation as can be seen from chromatogram 2B and 2E. (ii) The chromatogram of the PG incubated AIS from the control (Fig. 2D) shows the largest peak at a retention time of 28 min, whereas that of the same sample not PG incubated has the largest peak at retention time 23 min. (iii) AIS from orange PE treated paste undergoes the largest change by PG treatment (compare Fig. 2C and F). This indicates that PG predominantly hydrolyzes the pectin subfractions which have been precipitated as a result of the orange PE hydrolysis.

Small quantities of pectins with a polymerization degree below 10 have been solubilized as a result of the hydrolyzing activity of PG (Table 3). Much less molecules with DP 6-10 than molecules with DP 1-5 have gone into solution. Largest quantities of oligomers (DP 1-10) are released from AIS of the fungal PE treated paste after PG treatment.

The results of HPSEC and HPIEC both indicate that trace amounts of PG, present in the fungal PE preparation had hydrolyzed part of the AIS pectin of the diluted tomato paste during incubation.

6.3.3 Effect of hydrolysis by PE enzymes on the rheological properties

Diluted paste was incubated either with fungal PE (50 U/g WIS) or orange PE (5 U/g WIS); the suspensions were incubated at 30 and 40 °C respectively. Changes in rheological properties were followed.

In Fig. 3 yield stress is depicted as a function of incubation time of both suspensions. Both enzyme treated diluted pastes formed stronger gels as followed from the strongly increasing yield stress. The yield stress of the orange PE (5 U/g WIS) treated paste increased about 4 times after 7 h of incubation, whereas that of fungal PE treated paste, incubated with a 10 times higher

dosage (50 U/g WIS), increased only 2 times. This difference must be due to the different modes of action of the two PE enzyme preparations. The block wise deesterification of orange PE resulted in a much more effective gel formation than the at random deesterification by fungal PE. In Table 1 it is shown that DE of fungal PE treated diluted paste decreases until 6.4%. According to Thibault and Rinaudo (1985) calcium cooperative binding remains negligible when more than 40% of the carboxyl groups are randomly esterified. Gel-forming ability increases with decreasing DE. The polymer should have at least seven non esterified residues occuring consecutively along the chain (Thibault and Rinaudo, 1985).

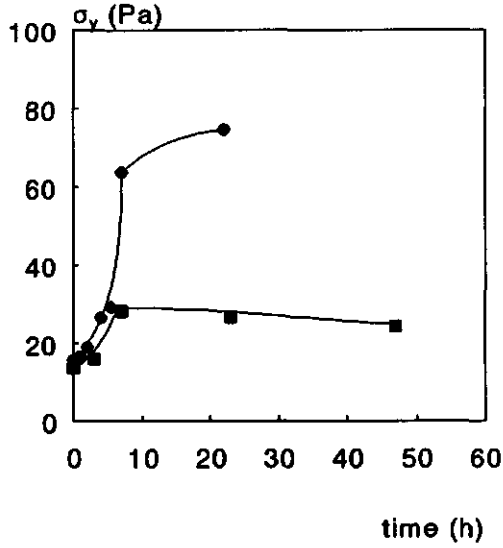

Figure 3. Yield stress as a function of incubation time for fungal PE (50 U/g WIS; ■) and orange PE (5 U/g WIS; ●) treated hot break diluted tomato paste.

Another difference in the rheological performance of both PE preparations is the behaviour of the yield stress for incubation times longer than 7 h. For the diluted paste incubated with orange PE, σ_y becomes slightly higher for longer incubation times whereas for the fungal PE, σ_y tended to become lower. This decrease is probably a result of the presence of some PG activity in the fungal PE enzyme preparation. Orange PE is free of PG, as can be concluded from Fig. 1. PG has a large effect on the rheological behaviour of tomato suspensions, it strongly decreases the apparent viscosity of diluted tomato paste (Foda and McCollum, 1970; Heutink, 1986).

Apparent viscosity measured at a shear rate of 86 s^{-1} is plotted as a function of incubation time in Fig. 4. For both enzymes there is only a minimal increase (less than 10%) of the apparent viscosity with incubation time. This observation is in agreement with that found by Heutink on a diluted 10 °Brix tomato concentrate incubated with fungal PE. Neither an increase, nor a decrease was

found by Heutink. The gel character and the high yield stress of the enzyme treated tomato suspensions seemed to be destroyed to a large extent by the shearing ($\dot{\gamma}$ = 86 s^{-1}). It did not restore after shearing within several hours. This means that the calcium pectinate gel structure had been destroyed irreversibly by the enforced shear.

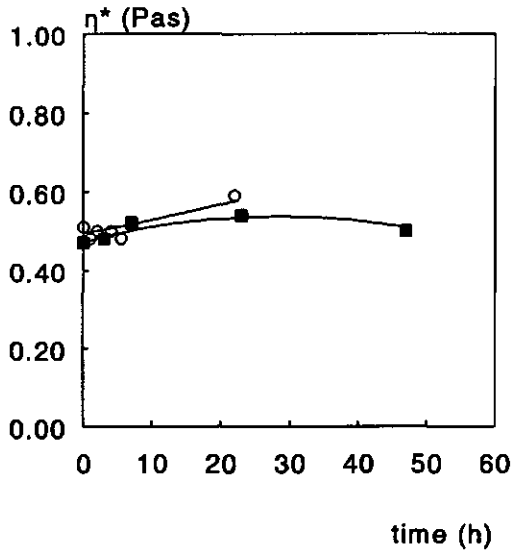

Figure 4. Apparent viscosity determined at a shear rate of 86 s^{-1} as a function of incubation time for fungal PE (50 U/g WIS; ■) and orange PE (5 U/g WIS; ○) treated hot break diluted tomato paste.

Serum viscosities as a function of incubation time measured at a temperature of 25 °C are given in Fig. 5. Sera of the two enzyme treated suspensions behave in a comparable way: a maximum serum viscosity was measured after about 3 h of incubation. The maximum was about 25% higher than the original serum viscosity. After about 7 h of incubation the serum viscosity decreased to about 50% of its original serum viscosity.

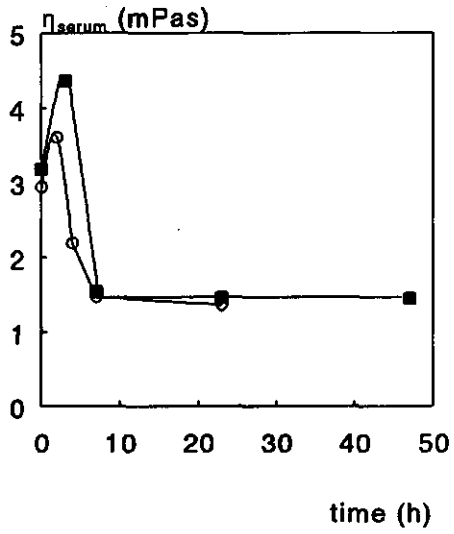

Figure 5. Serum viscosity as a function of incubation time of fungal PE (50 U/g WIS; ■) and orange PE (5 U/g WIS; ○) treated hot break diluted tomato paste (temperature = 25 °C).

When the sera were cooled down from 25 °C to 5 °C, the serum of the 3 h fungal PE treated diluted paste behaved as a gel. This is illustrated in Fig. 6, in which *G'* of the serum is followed as a function of time at 5 °C. The other sera did not show such a gelling behaviour. By raising the temperature to 20-25 °C the gel "melted" and it was reformed when the temperature was lowered again to 5 °C. So the gel had a thermally reversible character. Probably a calcium-pectinate gel is formed at low temperatures. The temperature dependency of calcium-pectinate gelformation has been demonstrated by Garnier et al (1993).

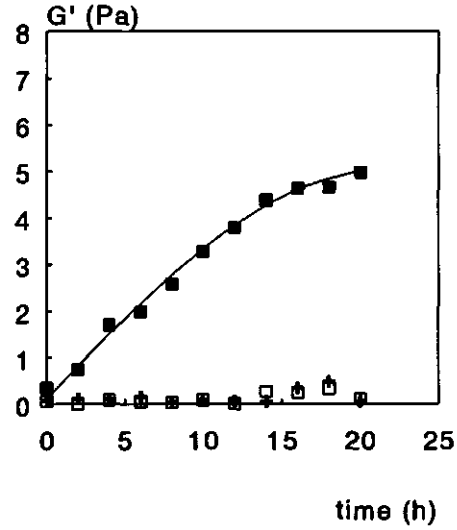

Figure 6. *G'* as a function of measuring time at 5 °C of various sera from fungal PE treated paste; control (+); incubation time 3 hours (■) and 7 hours (□); maximum strain = 2.10^{-3}, frequency = 0.15 Hz.

6.3.4 The effect of homogenization on orange PE treated tomato suspensions

Hot break diluted tomato paste was incubated with orange PE at three levels (0, 5 and 20 U/g WIS respectively) during 4 h at 40 °C. After the incubation the suspension was mixed with other ingredients and subsequently homogenized at a temperature of 85 °C. Non homogenized samples showed a slight increase of apparent viscosity due to the enzyme treatment, which is in agreement with other results given above. The relatively small increase of yield stress was caused by the mixing step after incubation, by which the calcium pectinate gel structure had been destroyed. For the determination of σ_y given in Fig. 3, mixing and shearing were limited as much as possible. After homogenization the rheological parameters of the enzyme treated samples were lower than for the control (Table 4 and Fig. 7).

Table 4. Yield stress (σ_y) and apparent viscosity (η^*) determined at $\dot{\gamma} = 85.7\ s^{-1}$, of orange PE treated samples, non homogenized and homogenized.

PE dosage (U/g WIS)	Non homogenized		Homogenized	
	σ_y (Pa)	η^* (Pas)	σ_y (Pa)	η^* (Pas)
Control	10.7	0.44	58.9	1.08
5 U	11.6	0.46	51.6	1.11
20 U	13.3	0.51	36.8	0.99

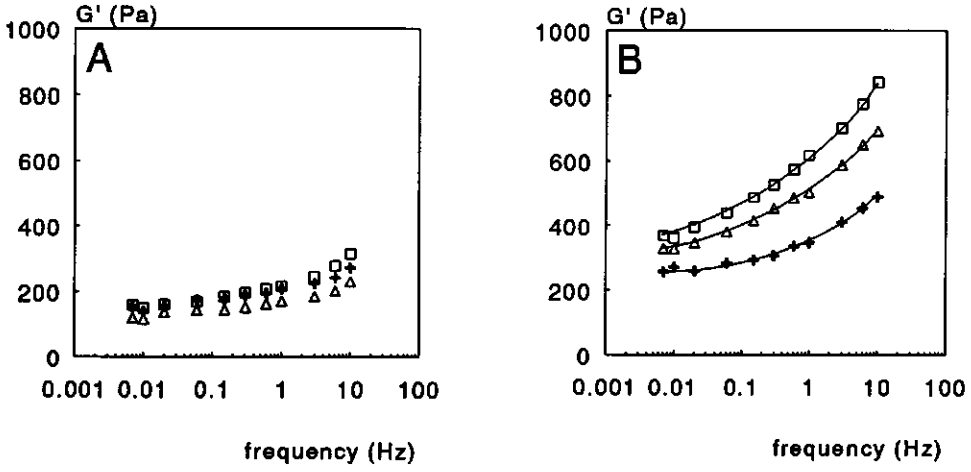

Figure 7. *G'* as a function of frequency for non-homogenized (**A.**) and homogenized (**B.**) diluted tomato samples; control (□), incubated with 5 U orange PE/g WIS (△) or 20 U orange PE/g WIS (+) for 4 h at 40 °C, prior to homogenization.

Estimated serum quantities of the homogenized samples are given in Table 5. It is clear from these numbers that the amount of serum increases with orange PE dosage.

Table 5. The amount of serum, formed on top of the product, of orange PE treated, homogenized tomato suspensions.

PE dosage (U/g WIS)	Average serum (g)
Control	1.7
5	6.3
20	7.2

6.4 Discussion

Both fungal and orange PE deesterify tomato pectin. The block wise deesterification by orange PE results in a relatively much faster calcium pectinate gel formation. In tomato suspensions, orange PE deesterifies in a blockwise manner predominantly the soluble highly esterified serum pectin. Cell walls also contain highly esterified pectin but it is probably more difficult for the enzyme to approach this substrate unless these pectins are located at the outside of the cell walls. Mostly the substrate is surrounded by other cell wall polymers. Due to the deesterification of the serum pectin, a calcium pectinate gel is formed, which is certified by the strong increase of the measured yield stress (Fig. 3). This calcium pectinate network forms an intercellular network. It is not clear whether the tomato cells and particles participate in the calcium pectinate network or form an independent network. Probably they form a part of the integral network due to the presence of the PE deesteried pectins from the middle lamella at the outside of the cell wall.

In order to get a calcium pectinate gel some requirements have to be met; (i) the concentration of low esterified pectin has to be high enough (ii) the calcium concentration must be high enough to get calcium bridges formed. Other factors playing a role in the gelling behaviour and gel strength are pH, ionic strength, soluble solids content and temperature (Axelos and Thibault, 1991; Garnier et al, 1993). In tomato products the calcium concentration is about 4 - 6 mmol/100 g tomato solids (Davies and Hobson, 1981) whereas the galacturonic acid concentration is about 20 mmol/100 g tomato solids. This means the Ca^{2+} concentration can be a critical factor for creating the calcium pectinate network (Axelos et al, 1992).

The higher WIS content (Table 1) and lower serum viscosity (Fig. 5) after long incubation times (>4 h) show that the calcium-pectinate precipitates onto the tomato cells. This precipitation reaction is probably caused by the heating and or shearing of the PE treated tomato cell suspensions. The situation described here can be compared to some extent with model experiments on calcium and low methoxylated pectin described by Axelos et al (1989) and Garnier et al (1992). These workers illustrated the combined influence of calcium and low methoxylated pectin concentration on the gelation of LM pectin by a phase diagram. Three regions could be distinguished: the sol phase, the gel phase and the phase where the gel is susceptible to syneresis. At long incubation times of PE incubated tomato suspensions, the concentration of low methoxylated pectin becomes that high that the gel probably becomes susceptible to syneresis which probably results in the precipitation of the calcium pectinate onto the tomato particles.

By homogenization of tomato suspensions the rheological parameters determined of the whole suspension (σ_y, η^*, G') increase. However, it is clear that for all these parameters the increase is lower for PE treated homogenized suspensions than for the non enzyme treated homogenized suspension. Homogenization results in a kind of transition of a network of round shaped cells into a network consisting of fibrous, hairy particles, in which the hairy cellulose fibres probably determine to a large extent the physical properties (chapter 4). Maybe the precipitated calcium pectinate stiffens the cell particles making it more difficult to transform them to fibrous, hairy particles by homogenization.

PE treated, homogenized tomato suspensions exhibited more serum separation as appears from Table 5. As serum separation is inversely proportional to G' (chapter 2 and 7) this can be explained by the lower value of G' (Fig. 7) of the enzyme treated homogenized suspensions. Furthermore, serum separation will be accelerated by the lower serum viscosity of the enzyme treated suspension. The influence of serum viscosity on the velocity of serum separation will be discussed in chapter 7.

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7 On the physical stability of fluid tomato products

Summary

The main physical instability of fluid tomato products is the formation of a serum layer on top of it. It may be caused by several mechanisms. Probably the primary origin is uniaxial compression of the weak particle network due to the gravitational force. This process continues until the sedimentation stress equals the compression modulus times the deformation gradient of the network. Less serum will be formed if the product adheres to the wall of the packaging. Packaging materials (polypropylene versus glass) differ in adhesion-cohesion behaviour towards fluid tomato suspensions. If there are differences in the level of the upper surface of the tomato product, serum formation may also be caused by drainage of serum from the tomato suspension above the lower level to the "valleys".

Potential height differences and resultant serum formation are related to e.g. the yield stress of the product. The velocity of serum separation can be described according Darcy's law. Permeability coefficients were determined and flow velocities in case of uniaxial compression and drainage were estimated. In the latter case anticipated flow velocities were much higher. Theoretical calculations indicate that for short storage time serum separation is mainly caused by drainage due to unevenness in the surface level and for long storage time due to uniaxial compression. It was found that a non homogenized tomato product with a water insoluble solids content of 1.5% showed less serum separation than a homogenized tomato product. This is in contrast with calculated quantities according to the uniaxial compression model. A possible explanation is that the non homogenized product adheres to the container whereas the homogenized product does not show this behaviour.

7.1 Introduction

Fluid tomato products may demix during storage resulting in the formation of e.g. a serum layer on top of the juice column (Robinson et al, 1956; Shomer et al, 1984). Tomato ketchup also exhibits the tendency to form a serum layer on top of the product (Stoforos and Reid, 1990 and 1992).

Several methods have been applied to measure the extent of serum separation. Robinson et al (1956) and Shomer et al (1984) determined serum separation by letting the juice stand to sediment over a certain time and measuring the serum layer formed. In the blotter test a ketchup sample is placed on a filter paper and the distance of serum flow on the paper is measured

(Nelson et al, 1957). Caradec et al (1985) constructed a 42 mesh steel screen in the shape of a cone with a 60 ° angle and ca 10 cm side length. A sample of tomato juice was put on it and the amount of serum was recorded. Stoforos and Reid (1990, 1992) measured serum formation by placing a small amount of ketchup on a wire screen mounted at the bottom of a plexiglass tube. Serum drained through and was collected. Except for the methods used by Robinson et al (1956) and Shomer et al (1984), these tests have in common that they were developed in order to have a fast method to measure serum separation. They are used as a predicting method for serum separation in a practical situation. However, the direction, magnitude and duration of the forces acting on the product during these tests are not compatible with the conditions under which serum separation occurs in practice. Therefore care should be taken with the interpretation of the results obtained by these tests.

According to Robinson et al (1956) who used the term settling in stead of serum separation, there is an inverse relationship between the degree of settling and the apparent viscosity of tomato juice. The degree of settling was influenced by the amount of suspended solids and the extent to which the intact cells were disrupted. Homogenization reduced the degree of settling.

Shomer et al (1984) explained the formation of serum in the uppermost region in a juice column by two distinct mechanisms. a: Too much liquid, so that a given concentration of insoluble particles cannot fill the volume of the liquid column; serum separation may occur within several hours of shelf life.
b: Slowly diminishing volume of the precipitate during storage as a result of gradual collapse under gravity stresses.
By ultrastructural observations they revealed that degradation of cell wall pectic substances, in which the cellulose microfibrils are embedded, enabled partial dispersion of the microfibrillar system and probably induced swelling of the cell wall. This led to a swollen precipitate with an increased volume and as a result reduced the amount of serum.

According to van Vliet and Walstra (1988) different types of demixing of liquid dispersions may be distinguished; 1. sedimentation or creaming, 2. syneresis, i.e. formation of a clear liquid layer; 3. separation, i.e. layer formation due to uniaxial compression of the network under its own weight. In the latter case adherence to the wall of the container may play a role in the stability of the product.

In this work the physical nature of serum separation of concentrated fluid tomato suspensions was studied by e.g. rheological methods and permeametry. Special attention was paid to the effect of homogenization.

7.2 Experimental

Preparation of tomato suspension

Tomato suspensions were prepared from an industrial hot break tomato paste (28 °Brix). The suspension was standardized on 1.5% water insoluble solids (WIS), 32.7 °Brix, 0.58 M NaCl and pH 3.4 (with acetic acid) and heated to 90°C. Part of the suspension was homogenized at a pressure of 17 MPa (Rannie, Denmark) at that temperature. Subsequently the suspensions were cooled to room temperature.

Permeability experiments

Permeability was measured according to the method developed and described by van Dijk (1982 and 1986). Glass tubes, open at two ends with a diameter of 4 mm and a length of 25 cm were filled with tomato suspension over a length of 6 cm. After filling the tubes were placed in a rack, which was placed in the measuring vat of plexiglass. The measuring vat contained artificial serum (η = 12.5 mPas, $\rho = 1.22 \cdot 10^3$ kgm^{-3}, pH 3.8). For the non homogenized suspension the initial height difference between tomato suspension level and serum level was about 1.5 cm, for the homogenized tomato suspension this was 3 cm. For the non homogenized and the homogenized suspension the initial pressure gradient was about $3 \cdot 10^3$ and $6 \cdot 10^3$ Pam^{-1} respectively. Each hour the level of serum in the tubes was registered.

In case Reynolds (*Re*) is small enough, which was the case in this system, Darcy's law for flow through a porous medium in one direction applies (1).

$$v = -(B/\eta)\nabla P \qquad (1)$$

Where is B (m^2) the permeability coefficient, v (m$\cdot$s^{-1}) the liquid flux of serum through the matrix of the tomato suspension, which is caused by the pressure gradient ∇P and η is the viscosity of the serum. B can be calculated from the applied ∇P and the measured liquid flow v and η. Variation of the pressure gradient ∇P (+ or - 30 %) did not influence the value of B observed, implying Darcy's law is applicable.

Rheological measurements

For all rheological measurements an equilibration time of 15 min was taken into account after bringing the sample in the rheometer. Rheological measurements were conducted at a temperature of 20 °C. In order to prevent slip sherated measuring bodies or the measuring bodies covered with emery paper were used.

The yield stress (σ_y) was measured with a Deer Rheometer PDR81 using parallel plates (diameter 5 cm) and a distance between the plates of 2 mm. A constant stress was applied and the strain rate was monitored. Every 10 min

the stress was increased by 5 or 10 Nm^{-2}. The minimum stress at which the apparent viscosity decreased suddenly a few orders of magnitude was taken as the yield stress.

Apparent viscosity (η^*) measurements, in which the shear stress (σ) was determined as a function of shear rate ($\dot{\gamma}$), were done with a Haake Rotovisco RV20, either fitted with a smooth coaxial cylinder MV/MV3 measuring system or with the coaxial cylinder measuring system, type P (ribbed outer cylinder) and a MV3 inner cylinder covered with sandpaper (cornsize 80, 0.02-0.04 mm).

Dynamic measurements were carried out with a Bohlin VOR rheometer, fitted with sherated plates (diameter 3 cm). The storage modulus G' was determined at a strain of $2 \cdot 10^{-3}$ and a frequency of 0.01 Hz. Serum viscosity was measured with an Ubbelohde capillary viscometer, after centrifuging the tomato suspension at 30.000 *g* in a Sorval RC-5B centrifuge (20 min) and filtering the serum through Schleicher and Schüll membrane filters (5 and 1 μm respectively).

Determining extent of adherence of tomato suspensions to a solid material

Clean plates from glass or polypropylene, which are commonly used as packaging materials for tomato products, dimensions 10 x 10 cm were dipped in the homogenized tomato product. Subsequently the amount of product that stuck to the plate was weighed as a function of time during half an hour. The procedure was repeated four times. The experiment was conducted under controlled humidity conditions.

Determining serum separation

Polypropylene bottles (ca 500 ml, radius 3 cm, filling height ca 0.1 m) were filled with circa 350 g of tomato suspension. The bottles were rotated over 360°, to simulate consumer's handling, and stored at 20 °C in the dark. The amount of serum formed on top of the product was determined after different time intervals by decanting the bottle and weighing the amount of serum.

7.3 Results

7.3.1 Rheological properties

The results of rheological measurements are given in Table 1. An extensive treatise about the effect of homogenization on the rheological properties is given in chapter 4. So only values relevant to the serum separation process are included. Serum separation is a result of a physical destabilizing process over a relatively long time scale. This implies that mainly rheological properties at

relatively low deformation rates and the serum viscosity are relevant.

Table 1. σ_y, G' (determined at 0.01 Hz), η_{serum}, and B for the non homogenized and homogenized tomato suspension.

	σ_y (Pa)	G' (Pa)	η_{serum} (mPas)	B (m^2)
Non homogenized	25	230	11	$9.3 \cdot 10^{-13}$
Homogenized	40	250	12	$1.9 \cdot 10^{-13}$

As can be seen in Table 1, G' of the homogenized product was about 10% higher than G' of the non homogenized product, which is in agreement with results given before (chapter 4).
The yield stress is related to the potential height difference in product level after a product is poured on a flat surface, according to

$$\sigma_y = \rho h g \qquad (2)$$

Where σ_y is yield stress, ρ is density of the suspension and g is gravitational acceleration, h is the potential difference in height. Using the values for σ_y given in Table 1, h can be estimated as 2 and 4 mm for the non homogenized and the homogenized product respectively.

7.3.2 Permeability

The measured permeability coefficient B for the non homogenized tomato product was $9.3 \pm 1.3 \cdot 10^{-13}$ m^2. The homogenized product had a 5 times lower value for B: $1.9 \pm 1.2 \cdot 10^{-13}$ m^2. B depends on the geometry, scale and spatial distribution of the porous matrix. In fact it is a measure of the number and size of the relatively large pores in the material. The lower value for B for the homogenized product must be a result of the change in structure during homogenization. It indicates that in the non homogenized suspension much larger pores are present than in the homogenized one.

7.3.3 Serum separation

In the non homogenized tomato product the amount of serum formed was minimal, in all cases less than 1 g (Fig. 1). However, the homogenized product showed much more serum separation, sometimes more than ten times the amount formed by the non homogenized products. The amount of serum also depends on storage time; after about 4 weeks storage the amount does not change anymore for the homogenized suspension. For the non-homogenized

product this was the case within one week, which is probably due to the relatively large permeability coefficient (*B*) of these suspensions. This will be discussed later.

A complication of a practical nature concerning the serum measurements of the non homogenized products was the poor distinction between serum and suspension by which it was hard to estimate the serum quantity. This is probably caused by the small particles which flow with the serum through the large pores of the network to the upper layers of the product. In the homogenized tomato product this process is hindered due to the much lower permeability, resulting in a clear distinction between serum and suspension.

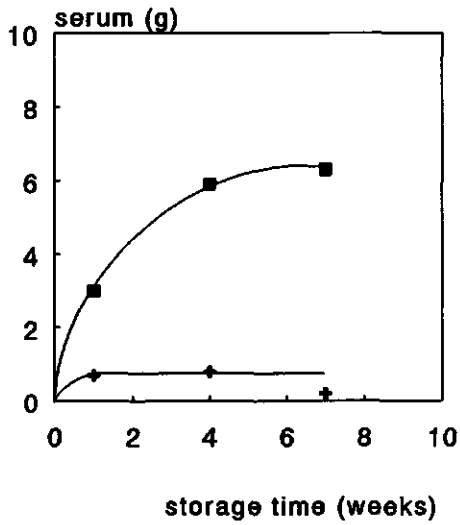

Figure 1. The amount of serum formed on top of the tomato product homogenized (■) and non homogenized (+), as a function of storage time.

7.3.4 Adherence of a tomato suspension to a solid material

Results are shown in Figure 2 A and B. As can be seen the materials tested, exhibited different behaviours with respect to "stickiness" to the tomato product. The tomato product adhered better to glass than to polypropylene. It is known that glass has a hydrophilic surface and therefore it is thought the interaction forces have an electrostatic character. It may be that negatively charged groups of the pectic molecules interact with positively charged groups of glass. Mizrahi and Berk (1970) proved that part of the cloud particles from orange juice have a negative charge. Moreover, it is remarkable that the product sticks

less to the plates after more times of dipping. It is likely there is some slippage due to the formation of a serum layer between product and plate. Adherence of a food poduct to solid material (i.e. glass or plastic) is a well known phenomenon (van den Boomgaard et al, 1987; Claassens, 1958 and 1959). The difficulty is the quantification of these forces. Methods described in literature to measure adhesion-cohesion forces (Claassens, 1958; Akkerman, 1992), are only suitable for determining interaction forces between solidlike food products (butter, curd) and solid materials (glass, plastic, steel). To date, no methods have been described for measuring interaction forces between fluid food products and solid materials. This is probably due to the complexity of the measurement.

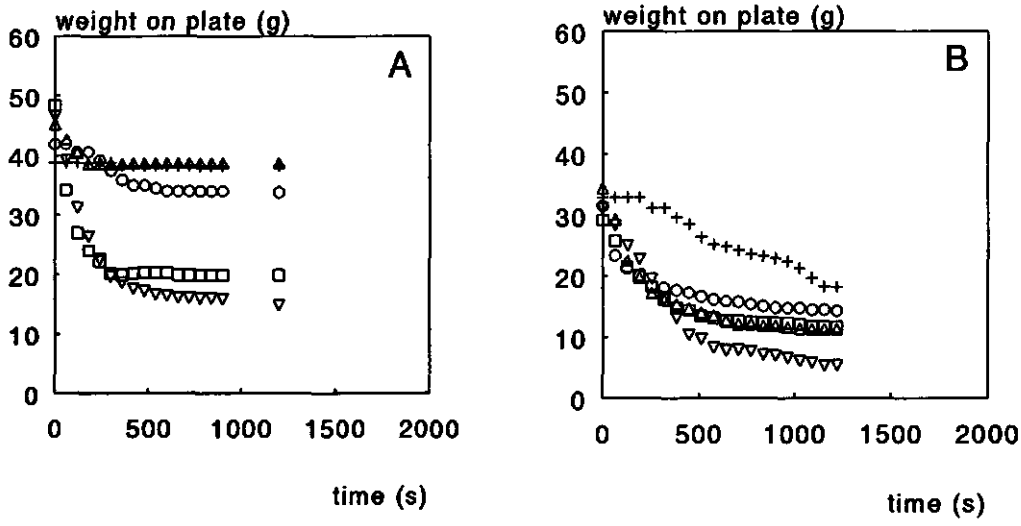

Figure 2. The amount of product that sticks to the plate as a function of time after the plate has been dipped in the homogenized tomato product **A.** = glass plate, **B.** = polypropylene plate. + = after 1st time, ▲ = after 2nd time, ○ = after 3rd time, ▽ = after 4th time and □ = after 5th time of dipping.

The reproducibility of the semi empirical test used for determining the interaction between the tomato products and the model packaging material was poor. However, the results clearly illustrate in a qualitative manner the difference in cohesion-adhesion behaviour between glass or polypropylene and a tomato suspension. Within the accuracy of the test no effect of different tomato products could be observed.

7.4 Discussion

One of the mechanisms which may lead to separation of tomato suspensions and so to serum formation is compression of the network of the insoluble tomato solids present in such products due to the gravitational force. This mechanism has been described in general by van Vliet and Walstra (1988). The network is compressed by its own weight because the fibre particles, making up the network have a higher density than the surrounding serum (Heutink, 1986; Siliha, 1985). This compression continues until the gravitational force is counteracted by the product of the compression modulus E of the network and the strain ϵ ($\sim \Delta h/h$, where h is the height of the network and Δh the difference in height due to the compression of the network) (van Vliet and Walstra, 1988). In formula:

$$E(\epsilon)\epsilon = \phi \Delta \rho g h \qquad (3)$$

in which ϕ is the volume fraction of suspended particles, $\Delta\rho$ the density difference between the network particles and the serum and g acceleration due to gravity.

This equation is valid in an ideal situation where there is no interaction of the network with the packaging material. If there is adherence of the product to the wall of the bottle the value of ϵ will be lower than the quantity calculated according to (3) because the effective h will be lower. The uniaxial compression modulus E is about 2-3 times the storage modulus (G') (Whorlow, 1992). The homogenized and the non homogenized suspension used in this study had a WIS content of 1.5%, which implies that $\phi\Delta\rho$ for both suspensions is the same. Therefore according to equation 3 one would expect that if there is no interaction between the network and the packaging material the amount of serum is related inversely to G'. Supposed there is an ideal situation, then G' of the tomato product would determine the amount of serum which is formed. As appears from Table 1, G' of the homogenized suspension is about 10% higher, therefore more or less the same amount of serum is expected for both suspensions. This is not in agreement with the amount of serum observed experimentally (Figure 1); the homogenized suspension exhibits much more serum separation.

Serum separation may also be caused by drainage when the upper surface of the product is uneven. This may happen i.e. in a half emptied bottle or when the product is poured on a plate. Potential height differences of product level are directly related to the yield stress (equation 2). As discussed above, level differences of 0.2 and 0.4 cm can be expected for the non homogenized and the homogenized suspension respectively. Suppose half of the surface (30 cm^2)

the homogenized suspension respectively. Suppose half of the surface (30 cm^2) in the bottle is above the average level and from that volume 50% of the liquid drains off, then ultimately about 1.5 and 3 g of serum will be formed for the non homogenized and the homogenized suspension respectively. This quantity has to be added to the quantity formed due to compression of the network (Table 2). In the calculations of the latter amount it was not taken into consideration that E increases strongly, more than in proportion to the increase in ϕ with ϵ, during compression. Therefore the numbers given in Table 2 should be considered as approximate values which are likely to be somewhat too high.

A similar reasoning as for the effect of unevenness of the surface can be given for the case that some tomato suspension is sticking to the packaging material above the bulk level.

Besides the total quantity of serum the rate at which it is formed, is also of importance in practice. The rate will be related to the velocity of the liquid flow through the porous network, which is determined by the permeability of the system. As discussed above the velocity can be calculated according to Darcy's law (equation 1). In tomato products ∇P is caused by the difference in density between the network and the continuous phase. Initially ∇P equals $\phi\Delta\rho g$. In case of compression $\phi\Delta\rho \approx 10$ kgm^{-3}, so ∇P is about 100 Pam^{-1}. By inserting the values observed for B and η_{serum} (Table 1) in equation 1, a value for v is obtained of $8.5 \cdot 10^{-9}$ and $1.6 \cdot 10^{-9}$ $m \cdot s^{-1}$ for the non homogenized and the homogenized tomato suspension respectively. This implies a separation velocity of initially 0.7 and 0.1 mm per day, respectively. Serum flows much easier through the spaces between whole cells of a non homogenized suspension, than through a matrix consisting of long shaped fibrous particles of the homogenized product.

In case of drainage ∇P is roughly 10^3-10^4 Pam^{-1}, so the initial separation velocity will be about 10-100 times faster than in case of compression. Initial velocity numbers as a result of compression and drainage are also summarized in Table 2.

As can be seen in Fig. 1 the homogenized suspension exhibits more serum formation than the non homogenized suspension which is contrary to theoretical expectations (Table 2). For the homogenized product the observed and the theoretical expected amounts are roughly in agreement but for the non homogenized product the observed amount is much less than predicted. An explanation for this discrepancy is that the non homogenized suspension adheres to the packaging material whereas the homogenized suspension does not. By adherence of the non homogenized suspension to the packaging material the effective h and therewith Δh will be much lower.

Table 2. Calculated values of Δh, serum by compression, serum by drainage, initial velocity of serum separation by compression and drainage. For explanation see text.

Product	Serum separation (calculated)			Initial velocity of serum separation (calculated)		
	Compression		Drainage	Compression		Drainage
	Δh (mm)	quantity (g)	quantity (g)	mm/ day	g/ day	mm/ day
non homogenized	1.7	5.1	1.5	0.7	2.1	7-70
homogenized	1.6	4.8	3	0.1	0.3	1-10

This difference in adherence behaviour can be explained by the fact that in a homogenized suspension a layer of serum is formed at the interface between packaging material and suspension whereas non homogenized suspension does not exhibit serum formation near the surface of the packaging material. Adherence to the packaging material is excluded by the formation of a serum layer between packaging and the tomato suspension.

The difference in adherence behaviour between the non homogenized product and the homogenized tomato suspension is illustrated by Figure 3, representing polypropylene bottles half filled with (A) non homogenized tomato suspension and (B) tomato suspension homogenized at a pressure of 17 MPa. Both suspensions had a WIS content of 1.5%. The pictures were taken one week after filling, the bottles with tomato suspension were filled on the same way as for the serum separation measurements. It is clear from this observation that the non homogenized tomato suspension shows much better adherence behaviour to the packaging material than the homogenized suspension.

The test in which polypropylene plates were dipped in a homogenized and a non homogenized tomato suspension gave, within the large inaccuracy of the test, about the same weight of tomato suspension adhering to the plates. However, for the non homogenized tomato suspension it was a more or less equally divided thin layer as shown by Fig. 3A, while for the homogenized tomato suspension it were just a few thick blobs adhering to the plate.

Another indication for the difference in adherence behaviour between non homogenized and homogenized tomato suspensions are the results of viscosity measurements with roughened and smooth concentric cylinder measuring bodies for the non homogenized and the homogenized tomato suspension (Fig. 4 A and B). For the homogenized suspension the curve of stress versus shear rate obtained with the roughened measuring bodies lies at significantly higher values than the one obtained with the smooth measuring bodies. In case of the non homogenized suspension the difference is considerably smaller. Probably in the homogenized suspension a layer of serum at the interface of the measuring bodies of the viscometer and the tomato suspension is formed causing the measured stress to be lower. Measurements with roughened measuring bodies are less sensitive towards serum formation at the product-measuring body interface.

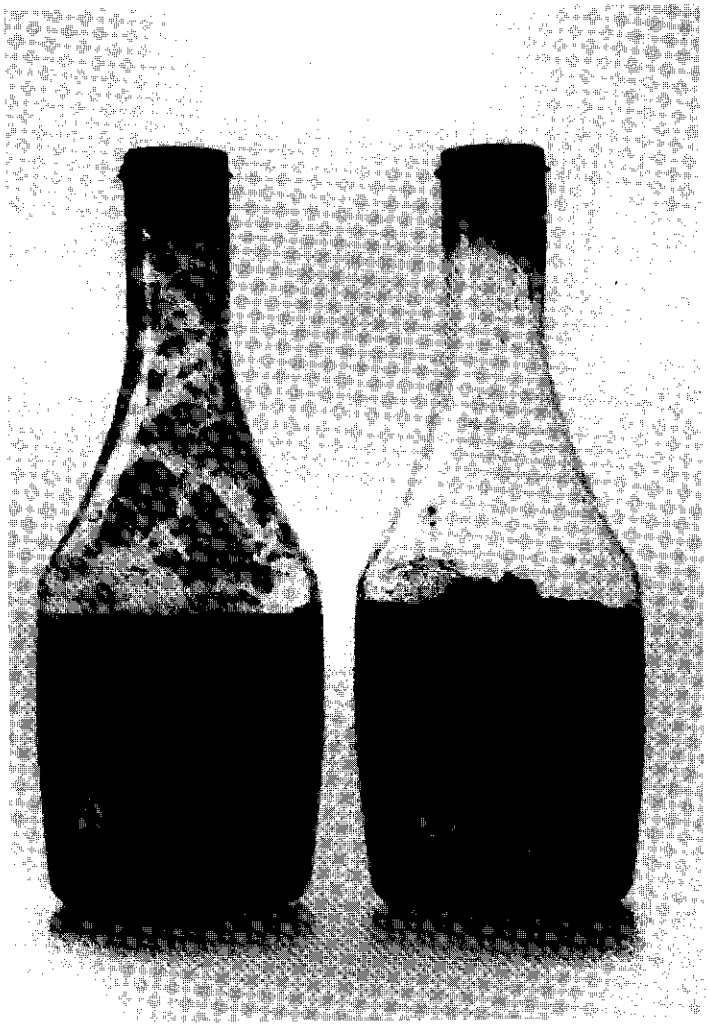

Figure 3. Polypropylene bottles filled with tomato suspension (storage time was one week). **A.** non homogenized, **B.** homogenized.

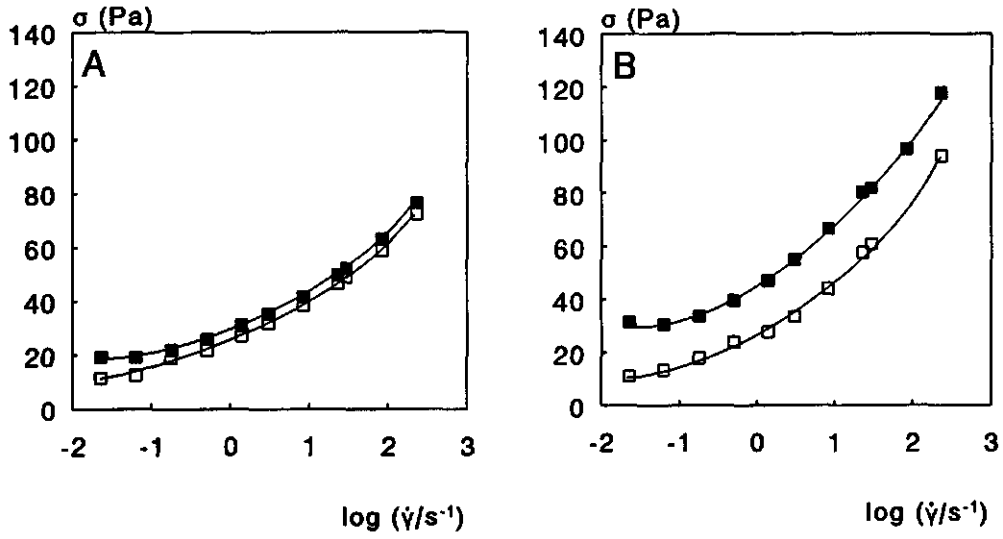

Figure 4. Shear stress (σ) as a function of shear rate ($\dot{\gamma}$) , as measured with a Haake rheometer fitted with the MV/MV3 (□) or the P/sandpaper covered MV3 (■) measuring system for **A.** a non homogenized tomato suspension and **B.** a homogenized (17 MPa) tomato suspension.

These results and those of the visual observations (Figure 3) form a strong circumstantial evidence for the fact that in a non homogenized tomato product serum separation is strongly reduced by adherence of the product to the packaging material. We come back on this later.

In chapter 2 it has been shown that homogenization of tomato suspensions with a concentration of water insoluble solids of 0.65% led to a three fold increase of G' and also to a significant reduction of the measured serum quantity (chapter 2). The very strong increase in G' upon homogenization for low WIS suspensions (0.5-07%) might explain why Shomer et al (1984) found lower quantities of serum for homogenized tomato juice than for non homogenized juice. Tomato juice has a WIS concentration of about 0.7%. At a WIS concentration of about 1.5% the increase in G' upon homogenization was found to be less pronounced; G' of the homogenized product was only 10% higher. Although no explicit research has been done on the combined effect of WIS concentration and homogenization on G', it is clear from these results that WIS

content may have a large influence on the effect of homogenization on G' and serum separation.

As shown in chapter 5 and 6 hydrolysis by polysaccharide degrading enzymes enhances serum separation. This indicates the importance of the chemical and physical structure of the tomato cell wall for serum separation. Below we will discuss the chemical and physical structure of the tomato cell wall in relation to the tendency of a tomato product to exhibit serum separation.

The tomato cell wall can be considered as a concentrated gel consisting of a biphasic system: a network of cellulose microfibrils embedded in a gellike matrix composed of pectic and hemicellulosic substances (Bacic et al 1988; van Buren, 1979). These two colloidal phases, each built up of macromolecules, are associated by covalent bonds or physico-chemical bonds as H-bonds, electrostatic interactions, calcium bridges and Van der Waals interactions (Fry, 1988 and 1989).

Cellulose is composed of long, linear chains of ß-(1-4)-glucosyl residues. The stiff macromolecules aggregate along their lengths by hydrogen bonds and Van der Waals interactions to form microfibrils (Dey and Brinson, 1984; Fry, 1988 and 1989). A single microfibril is estimated to consist of 60-70 D-glucan chains, resulting in estimated cross-section dimensions of 4.5 x 8.5 nm. Estimation of the degree of polymerization of the cellulose molecules is complicated by the necessity of first solubilizing the D-glucans, this process probably breaks the chains. The aggregated D-glucans within a fibril are so ordered that they are in fact crystalline (Dey and Brinson, 1984). The network consisting of cellulose fibrils is probably held together by xyloglucan molecules which are connected by hydrogen and Van der Waals bonds to the cellulose fibrils (Carpita and Gibeaut, 1993; Fry, 1989). In the tomato cell the cellulose microfibrils are responsible for the strength of the cell wall. As cellulose molecules interact tightly with each other to form microfibrils, relatively little water molecules will be bound by the cellulose molecules. The cellulose fibrils have a compact structure. An important property of cellulose fibrils with respect to the formation of serum is the relatively high density of these fibrils; the rather high density of the whole tomato cell is probably a result of the relatively high density of the cellulose microfibrils. In principle a dispersion of only cellulose fibrils is instable: separation will occur.

The other phase, consisting of hemicellulosic and pectic substances is a highly hydrated, amorphous phase. The macromolecules from this phase are either helical like homogalacturonan from the pectic fraction and protein (extensin), or may contain side branches like most polymers from the hemicellu-

lose fraction. Because they are flexible and/or branched, they do not form aggregates of stretched molecules like the cellulose molecules, but entrap relatively large amounts of water. Once extracted from the cell wall, a solution of these macromolecules is stable; they do not segregate or precipitate (Fry, 1989).

In an intact tomato cell both fractions form a biphasic gel, a network. However, its stability is effected by external influences either physically (homogenization), chemically or enzymatically.

Homogenization induces large pressure gradients (chapter 4) resulting in macroscopic fracture of the gellike cell wall. This probably also disturbs the association of the amorphous phase with the cellulosic fibrils. The two fractions, different in physical behaviour, are partly torn from each other and may under certain circumstances exhibit a tendency to segregate on microscale. This can be considered as a kind of thermodynamic incompatibility (Tolstoguzov, 1993). This local segregation results in the formation of small channels of 10-100 μm in a tomato suspension. Such channels or pores have been observed visually and microscopically in the homogenized tomato product. These channels in the network result in an inhomogeneous product in which serum separation is increased. The tendency to segregate also may explain why serum is formed at interfaces of solid materials and tomato suspension (see above).

Enzymes like pectin esterase (chapter 6) and other polysaccharide degrading enzymes (e.g. endo-arabinase and endo-glucanase: chapter 5) clearly enhanced serum separation. These enzymes are predominantly active on the macromolecules of the amorphous phase. Enzymatic hydrolysis of polymers from the amorphous phase could disturb the association of the two phases whereby the cellulose fibrillar network segregates to some extent from the amorphous phase. Such an enhanced segregation may explain that serum separation in these enzyme treated homogenized tomato suspensions is much stronger than in the non enzyme treated, homogenized tomato suspensions.

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Summary

When tomatoes are processed into tomato juice, tomato paste or other tomato based products, the tomatoes and tomato suspensions are subjected to physical processes like e.g. heating, finishing, concentration and homogenization. During these processes physical, chemical and enzymatic changes occur in the tomato suspensions. The research described in this thesis has been conducted in order to obtain fundamental knowledge about changes in physico-chemical properties of tomato suspensions as a result of some of the above mentioned processes.

Chapter 1 describes compositional features of the tomato, special attention has been paid to the cell wall composition, cell wall models and the pectolytic enzymes. A very concise survey of the rheological aspects of tomato products is given as well.

In chapter 2 some general rheological techniques which have been applied to characterize the physico-chemical structure of tomato products are presented. Besides experimental results on the effect of concentration are given. Overshoot experiments at low shear rate proved to be a good method to estimate "yield stresses". Tomato suspensions with standardized total tomato solids (TS) level were prepared from various concentrates with respect to refractive index. The refractive index (°Brix) of the tomato concentrate, from which tomato suspensions were prepared, had a large effect on the apparent viscosity and storage modulus. The apparent viscosity of a tomato suspension prepared from a 30 °Brix tomato concentrate was only 35% of that of a suspension prepared from a 4.9 °Brix juice, both standardized at the same level water insoluble solids and total tomato solids. The same trend was found for dynamic moduli. Microscopic fracture of the cellulosic microfibrillar network during concentration may be a reason for the lower values of rheological parameters in the suspension made of the more concentrated concentrate. Moreover, in the suspensions the average diameter of the tomato particles became smaller due to the concentration process which may also result in a lower apparent viscosity and storage modulus. After homogenizing the difference in apparent viscosity and storage modulus between the extremes was only about 10-15%.

Chapter 3 presents the results of a particle size distribution analysis of tomato paste by wet sieving. The bulk of the particles had a size between 45 and 180 μm and predominantly consisted of parenchyma cells. The wet sieve fractions were investigated by microscopy and by a laser diffraction method. Both methods showed that the size of many particles was significantly larger (up to 2-3 times) than the diameter of the pores through which they had passed during wet sieving. These results illustrate the strongly deformable character of the tomato cell wall. The 90-125 and 125-180 μm wet sieve fractions had

highest apparent viscosity and yield stress, before as well after homogenization. The >250 μm wet sieve fraction had lowest apparent viscosity and also exhibited the smallest viscosity increase upon homogenization. This fraction appeared to consist mainly of seed and skin fragments.

Chapter 4 describes the effect of homogenization on rheological properties and particle size and shape of tomato products, apple sauce and strawberry sauce. As a result of the homogenization treatment, the round shaped cells from tomato suspensions changed into much smaller fibrous, hairy particles. The apparent viscosity, yield stress and storage modulus increased upon homogenization. This is probably due to the strong friction between the fibrous, hairy particles by which they form a kind of clusters. Tan δ decreased somewhat due to homogenization, which indicates that the network obtained a more elastic character. The cell morphology of and the effect of homogenization on strawberry cells showed similarities with those of tomato cells. However, homogenization of apple sauce resulted in a decrease of the apparent viscosity. Larger, much more regular, non fibrous particles were formed, which slide much easier along each other than the hairy, fibrous particles in homogenized tomato suspensions. The difference in behaviour between tomato cells and apple cells is probably caused by differences in crystalline cellulose content and in cell wall structure especially the microfibrillar cellular structure, as was indicated by polarized light microscopy.

Chapter 5 and 6 both deal with the enzymatic modification of tomato suspensions. In chapter 5 the results are presented for tomato suspensions treated with highly purified enzymes of fungal origin: rhamnogalacturonase (RG), rhamnogalacturonan acetylesterase (RGAE), endo-arabinase (EA) and two endo-glucanase types (EG-a and EG-b). The first three enzymes are mainly active on the pectin fraction, especially the hairy regions of it. Depending on their specific mode of action fungal endo β-(1-4)-glucanases hydrolyze the internal β-(1-4)-linkages of cellulose derivatives, insoluble cellulose fibrils and xyloglucans. The decrease in water insoluble solids (WIS) due to the enzyme treatment after 8h was 7.2%, 3.6%, 6.2% and 3.6% for the tomato suspensions treated with RG/RGAE, EA, EG-a and EG-b respectively. Due to the enzyme treatment the tomato cells and particles disintegrate into smaller particles as was shown by laser diffraction particle size measurements. Values of rheological parameters decreased due to enzymatic hydrolysis. Treatment with EA and EG-b resulted in the largest decrease in apparent viscosity, serum viscosity, yield stress and storage modulus. Homogenization of the enzyme treated suspensions caused drastic changes in the rheological parameters. Enzyme treated diluted pastes which had lowest values for yield stress and apparent viscosity before homogenization had highest values for these

rheological parameters after homogenization. The storage modulus of all samples was about the same after the homogenization treatment. The enzyme preparations studied gave rise to more serum separation in the homogenized tomato suspensions.

In chapter 6 the effects of pectin esterase from orange and from fungi are presented. Both esterase types caused a strong increase of the yield stress. However, the block wise deesterification induced by orange PE caused a much stronger increase than the random deesterification by fungal PE. The increased yield stress is probably due to the formation of a calcium pectinate network. The apparent viscosity measured at a shear rate of 86 s^{-1} of the PE treated samples was only slightly higher. By shearing the calcium pectinate network is destroyed to a large extent. During PE action, serum viscosity increased initially with circa 25% to a maximum, after which it decreased to approximately 50% of the original value. This decrease is probably caused by the precipitation of the calcium pectinate onto the tomato particles resulting in a higher water insoluble solids content. Homogenized orange PE treated, tomato suspensions had lower apparent viscosities, yield stresses and values for G' than non enzyme treated, homogenized tomato suspensions. The PE incubated homogenized tomato suspensions exhibited more serum separation.

Chapter 7 deals with the physical stability of fluid tomato products. The main physical instability of fluid tomato products is the formation of a serum layer on top of it. It may be caused by several mechanisms. Probably the main origin is uniaxial compression of the weak particle network due to the gravitational force. This process continues until the sedimentation stress equals the compression modulus times the deformation gradient of the network. Less serum will be formed if the product adheres to the wall of the packaging. If the level of the upper surface of the tomato product is uneven, serum formation may also be caused by drainage of serum from the tomato suspension above the lower level to the "valleys". Potential height differences and therewith in serum formation are related to e.g. the yield stress of the product. The velocity of serum separation can be described according Darcy's law. To get a rough idea of the velocity the permeability coefficient was determined. Flow velocities in case of uniaxial compression and drainage were estimated. In the latter case anticipated flow velocities were much higher. Theoretical calculations indicate that for short storage time serum separation is probably mainly caused by drainage due to unevenness in the surface level and for long storage time due to uniaxial compression. It was found that a non homogenized tomato product with a water insoluble solids content of 1.5% showed less serum separation than a homogenized tomato product, with the same WIS concentration. This is in contrast with calculated quantities according to the uniaxial compression model.

An explanation is that the non homogenized product adheres to the wall of the bottle, whereas the homogenized product does not.

The physical behaviour of tomato suspensions can be better understood by considering the tomato cell wall as a concentrated, composite gel, consisting of cellulose microfibrils embedded in a matrix composed of pectic and hemicellulosic substances. Cellulose is composed of long, linear chains of β-(1-4)-glucosyl residues. The stiff macromolecules aggregate along their lengths by hydrogen bonds and Van der Waals interactions to form microfibrils. In a tomato cell wall the cellulose microfibrils are to a great extent responsible for the strength of the wall. Little water is bound by the cellulose microfibrils. The cellulose fibrils have a rather high density and are therefore primarily responsible for serum separation of the tomato cell suspensions. The second phase, the "jelly" phase, consisting of hemicellulosic and pectic substances, is a highly hydrated, amorphous phase. Large amounts of water are entrapped by this phase. The amorphous phase contributes to the stability of the tomato cell suspension against serum separation. In an intact tomato cell, both fractions are forming an intact biphasic gel. However, its stability and that of a tomato cell suspension are affected by external influences which can be e.g. physically, chemically or enzymatically. By homogenization the "jelly" phase and the cellulosic phase are partly torn from each other, by which these phases may exhibit the tendency to segregate on microscale. Enzymatic hydrolysis may lead to locally a kind of phase segregation which gives larger capillaries and so both enhanced and more serum separation.

Samenvatting

Wanneer tomaten verwerkt worden tot tomatensap, tomatenpasta of andere tomatenprodukten, worden de tomaten en tomatensuspensies onderworpen aan fysische processen zoals bijv. verhitten, zeven, concentreren en homogeniseren. Tijdens deze processen vinden er zowel fysische, chemische als enzymatische veranderingen plaats in de tomatensuspensies. Het in dit proefschrift beschreven onderzoek is uitgevoerd om basiskennis te verkrijgen omtrent veranderingen in de fysisch-chemische eigenschappen van tomatensuspensies als gevolg van enkele van de hierboven genoemde processen.

Hoofdstuk 1 beschrijft de samenstelling van de tomaat. Speciale aandacht wordt besteed aan de celwandsamenstelling, celwandmodellen en de pektolytische enzymen. Tevens wordt een beknopt overzicht gegeven van de reologische aspecten van tomatenprodukten.

In hoofdstuk 2 worden enkele algemeen bekende reologische technieken gepresenteerd, die gebruikt zijn om de fysisch-chemische structuur van tomatenprodukten te karakteriseren. Daarnaast worden de experimentele resultaten van het effect van concentreren gegeven. Overshoot metingen bij lage afschuifsnelheid bleken een goede manier te zijn om de "zwichspanning" te schatten. Er werden tomatensuspensies gemaakt met een gestandaardiseerde hoeveelheid tomatenbestanddelen van tomatenconcentraten met oplopende refractieindex. De refractieindex (°Brix) van het tomatenconcentraat, waarvan de tomatensuspensie gemaakt was, had een groot effect op de schijnbare viscositeit en de opslagmodulus van de suspensie. De schijnbare viscositeit van de tomatensuspensie bereid van een 30 °Brix tomatenconcentraat was slechts 35% van die van een suspensie gemaakt van een 4.9 °Brix sap, beide gestandaardiseerd op hetzelfde WIS gehalte. Dezelfde trend werd gevonden voor de opslagmodulus G'. De lagere waarden van de reologische parameters werden mogelijk veroorzaakt door microscopische breuk van het microfibrillaire cellulose netwerk, wat optreedt tijdens het concentreren. Bovendien zijn de lagere schijnbare viscositeit en G' waarschijnlijk gerelateerd aan de kleinere gemiddelde diameter van de tomatendeeltjes a.g.v. het concentratieproces. Na homogeniseren waren de verschillen in schijnbare viscositeit en G' tussen de extremen nog slechts 10-15%.

Hoofdstuk 3 toont de resultaten van de analyse van de deeltjesgrootteverdeling in een tomatenpasta d.m.v. natzeven. Het grootste deel van de deeltjes heeft een diameter tussen de 45 en de 180 μm en bestaat hoofdzakelijk uit parenchymcellen. De natzeeffracties werden onderzocht m.b.v. microscopie en een methode gebaseerd op laserdiffractie. Beide methoden lieten zien dat de diameter van veel deeltjes significant groter is (tot 2-3 maal) dan de diameter

van de openingen waardoor de deeltjes gegaan zijn tijdens natzeven. Deze resultaten illustreren dat de tomatencelwand makkelijk vervormbaar is. De 90-125 μm en 125-180 μm natzeeffracties hadden de hoogste schijnbare viscositeit en zwichtspanning, zowel voor als na homogeniseren. De natzeeffractie >250 μm had de laagste schijnbare viscositeit en vertoonde tevens de geringste viscositeitstoename a.g.v. homogeniseren. Deze fractie bestond voornamelijk uit fragmenten van pitten en van de schil.

Hoofdstuk 4 beschrijft het effect van homogeniseren op de reologische parameters, de deeltjesgrootte en de deeltjesvorm van tomatenprodukten, appelmoes en aardbeiensaus. Ten gevolge van de homogenisatiebehandeling veranderen de ronde tomatencellen in veel kleinere fibrillaire, harige deeltjes. De schijnbare viscositeit, zwichtspanning en opslagmodulus namen hierdoor toe. Dit is waarschijnlijk het gevolg van de sterke frictie tussen de fibrilachtige, harige deeltjes waardoor de deeltjes klusters met elkaar vormen. Tan δ nam iets af als gevolg van de homogenisatiebehandeling, hetgeen duidt op een toename van het elastische karakter van het netwerk. Aardbeicellen vertonen grote gelijkenis met tomatencellen voor wat betreft celmorfologie en het effect van homogeniseren. Homogenisatie van appelcellen daarentegen resulteert in een afname van de schijnbare viscositeit. Dit wordt waarschijnlijk veroorzaakt doordat meer regelmatige, grotere niet-fibrilachtige deeltjes gevormd worden die veel makkelijker over elkaar heen glijden dan de harige, fibrilachtige deeltjes van gehomogeniseerde tomatensuspensies. Het verschil in gedrag tussen tomaten- en appelcellen wordt waarschijnlijk veroorzaakt door verschillen in celwandstructuur en dan vooral de microfibrillaire cellulosestructuur.

Zowel hoofdstuk 5 als hoofdstuk 6 gaan over de enzymatische modificatie van tomatensuspensies. In hoofdstuk 5 worden de resultaten getoond van tomatensuspensies die behandeld zijn met gezuiverde enzympreparaten afkomstig van schimmels: rhamnogalacturonase (RG), rhamnogalacturonan acetylesterase (RGAE), endo-arabinase (EA) en twee endo-glucanase typen (EG-a en EG-b). De eerste drie enzymen zijn vooral actief op de pektinefractie en dan met name de "hairy regions" ervan. Afhankelijk van hun specificiteit hydrolyseren endo β-(1-4)-glucanasen de interne β-(1-4)-bindingen van cellulosederivaten, onoplosbare cellulosefibrillen en van xyloglucanen. Na 8 uur incubatie was de afname in het gehalte aan wateronoplosbare bestanddelen ten gevolge van de enzymbehandeling met RG/RGAE, EA, EG-a en EG-b resp. 7.2%, 3.6%, 6.2% en 3.6%. Aangetoond werd dat uit de tomatencellen kleine deeltjes vrijgemaakt werden. Ten gevolge van de enzymatische hydrolyse namen de schijnbare viscositeit, serumviscositeit, zwichtspanning en opslagmodulus af. Incubatie met EA en EG-b resulteerden relatief gezien in de grootste afname van de bovengenoemde reologische parameters. Homogeniseren van de

enzymbehandelde suspensies gaf een drastische stijging van de reologische parameters te zien. De enzymatisch behandelde verdunde tomatenpasta's die de laagste waarden hadden voor zwichtspanning en schijnbare viscositeit vóór homogeniseren hadden de hoogste waarden voor deze reologische parameters ná homogeniseren. De opslagmodulus van alle monsters was ongeveer gelijk na de homogenisatiebehandeling. De geteste enzympreparaten gaven aanleiding tot meer serumvorming van het na incubatie gehomogeniseerde produkt.

In hoofdstuk 6 worden de effecten van pektine-esterase (PE) van sinaasappel en van schimmel beschreven. Beide pektine-esterase typen veroorzaakten een sterke toename van de zwichtspanning. Echter, de bloksgewijze ontestering veroorzaakt door sinaasappel PE gaf aanleiding tot een veel sterkere toename van de zwichtspanning dan de "at random" ontestering door schimmel PE. De sterk toegenomen zwichtspanning is waarschijnlijk het gevolg van de vorming van een calciumpektinaat netwerk. De schijnbare viscositeit gemeten bij een afschuifsnelheid van 86 s^{-1} van de PE behandelde monsters was slechts fractioneel hoger. Door het afschuiven wordt het calciumpektinaat netwerk in grote mate teniet gedaan. Gedurende de PE incubatie, nam de serumviscositeit aanvankelijk toe met 25% tot een maximum, waarna deze afnam tot ongeveer 50% van de oorspronkelijke waarde. Deze afname is waarschijnlijk het gevolg een neerslagreactie van het calciumpektinaat op de tomatendeeltjes, hetgeen bevestigd kon worden door het toegenomen WIS gehalte. Sinaasappel PE behandelde, gehomogeniseerde tomatensuspensies hadden een lagere schijnbare viscositeit, zwichtspanning en G' dan de niet enzymbehandelde, gehomogeniseerde tomatensuspensies. Tevens werd aangetoond dat de sinaasappel PE behandelde, gehomogeniseerde tomatensuspensies meer serumseparatie vertoonden.

Hoofdstuk 7 gaat over de fysische stabiliteit van vloeibare tomatenprodukten. Het belangrijkste fysische instabiliteitsfenomeen van vloeibare tomatenprodukten is de vorming van een serumlaagje bovenop het produkt. Verschillende mechanismen kunnen daarvan de oorzaak zijn. De hoofdoorzaak is waarschijnlijk uniaxiale compressie van het zwakke deeltjesnetwerk a.g.v. van de zwaartekracht. Dit proces gaat door totdat de sedimentatiespanning gelijk is aan het produkt van de compressiemodulus en de vervormingsgradiënt van het netwerk. Er wordt minder serum gevormd indien het produkt aan de wand van de verpakking blijft plakken. Indien er niveauverschillen zijn in het oppervlak van het tomatenprodukt, zal serumvorming ook veroorzaakt kunnen worden door drainage van serum uit de hoger gelegen delen naar de "dalen". Potentiële hoogteverschillen en daarmee verschillen in serumvorming zijn gerelateerd aan de zwichtspanning van het produkt. De snelheid van serumvorming kan worden beschreven volgens de wet van Darcey. Voor het afschatten van de snelheid

werd de permeabiliteits-coefficiënt bepaald. Een schatting van de serumvormingssnelheden is gemaakt voor uniaxiale compressie en drainage. In het laatste geval waren de berekende snelheden een stuk groter. De theoretische berekeningen tonen tevens aan dat voor korte bewaartijden serumvorming waarschijnlijk hoofdzakelijk veroorzaakt wordt door ongelijkheid van het oppervlak terwijl bij langere bewaartijden de serumvorming vooral door uniaxiale compressie wordt veroorzaakt. Er werd aangetoond dat een niet gehomogeniseerde tomatensuspensie met een WIS gehalte van 1.5%, minder serumvorming geeft dan een gehomogeniseerde tomatensuspensie. Dit is in tegenstelling tot de waarden die berekend zijn met het uniaxiale compressiemodel. De mogelijke verklaring is dat ongehomogeniseerd produkt aan de wand van de fles blijft "plakken", terwijl het gehomogeniseerde produkt dit niet doet.

Het fysische gedrag van tomatensuspensies kan beter begrepen worden door de tomatencelwand te beschouwen als een geconcentreerd gel, bestaande uit cellulose microfibrillen welke weer in een geleiachtige matrix van pektine en hemicellulose bestanddelen liggen. Cellulose bestaat uit lange ketens van β-(1-4)-glucosyl eenheden. De stijve macromoleculen aggregeren via waterstofbruggen en Van der Waals interacties tot microfibrillen. De cellulosefibrillen hebben een hogere dichtheid dan het serum en worden daarom verantwoordelijk geacht voor serumseparatie van de tomatencelsuspensies. De tweede fase, de geleiachtige fase, bestaande uit hemicellulose en pektine bestanddelen, is een sterk gehydrateerde, amorfe fase. Relatief grote hoeveelheden water worden vastgehouden door deze fase. Deze amorfe fase draagt bij aan de stabiliteit van de tomatencelsuspensie tegen serumseparatie. In een intacte tomatencel vormen beide fasen een geheel, een twee-fasen netwerk. Echter, de stabiliteit ervan en dat van een tomatencelsuspensie wordt beïnvloed door fysische, chemische of enzymatische modificaties. Door homogenisatie worden de geleiachtige fase en de cellulose fase deels van elkaar gerukt, waardoor deze fasen de neiging hebben op microschaal te segregeren. Enzymatische hydrolyse leidt lokaal tot een soort faseschеiding hetgeen resuleert in grotere capillairen en daarmee versnelde en meer serumseparatie.

List of symbols

B	permeability coefficient	m^2
d_{10}	number-weighted mean diameter	m
d_{32}	surface-weighted mean diameter	m
E	uniaxial compression modulus	Nm^{-2}
f	frequency	s^{-1}
g	acceleration due to gravity	$m \cdot s^{-2}$
G'	storage modulus	Nm^{-2}
G''	loss modulus	Nm^{-2}
h	height	m
P	pressure	Nm^{-2}
∇P	pressure gradient	Nm^{-3}
Re	Reynolds number	
T	temperature	K, °C
tan δ	loss tangent	
TS	total tomato solids	
v	liquid flux	$m \cdot s^{-1}$
WIS	water insoluble solids	
x	eddy size	m
γ	shear strain	
$\dot{\gamma}$	shear strain rate	s^{-1}
δ	loss angle	rad
ϵ	relative deformation	
ϵ	power density	$Nm \cdot s^{-1}$
η	viscosity	$Nm^{-2}s$
η^*	apparent viscosity	$Nm^{-2}s$
η_{serum}	serum viscosity	$Nm^{-2}s$
ρ	mass density	kgm^{-3}
σ	shear stress	Nm^{-2}
σ_f	fracture stress	Nm^{-2}
σ_y	yield stress	Nm^{-2}
ϕ	volume fraction	
ω	angular frequency	$rads^{-1}$

Curriculum Vitae

De auteur werd geboren op 24 januari 1961 te Kockengen (nu gemeente Breukelen). Na het behalen van het VWO diploma aan de Rijksscholengemeenschap Minkema te Woerden werd in 1979 begonnen met de studie levensmiddelentechnologie aan de toenmalige Landbouwhogeschool te Wageningen. In 1986 werd het doctoraaldiploma behaald met de hoofdvakken levensmiddelennatuurkunde en zuiveltechnologie en als bijvak proceskunde. Aansluitend trad hij in dienst bij DMV-Campina BV te Veghel (thans Campina-Melkunie BV), bij de afdeling produktontwikkeling. Sinds juni 1989 is hij werkzaam bij Heinz BV te Elst. In dit dienstverband werd aan de Landbouwuniversiteit te Wageningen van oktober 1990 tot juni 1994 het in dit proefschrift beschreven onderzoek uitgevoerd.