

Factors contributing to the rheology of tomato puree

by

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**Thesis submitted to the University of Nottingham for the degree of
Doctor of Philosophy**

September 2012

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ABSTRACT

Tomato (*Solanum lycopersicum*) puree is created by homogenising the flesh of tomato fruits. The viscosity of this material and the fibrous content is of commercial interest. Tomato puree consists of suspended particles (consisting of whole cells, broken cells and cellular fragments) in an aqueous serum. The contribution of the non-soluble and soluble material to tomato puree rheology was studied with reference to the varying composition of solids and firmness of tomato fruit at four stages of ripeness; mature green, breaker, pink and red ripe. When purees from the red ripe and the breaker fruit with initial total solids contents of approximately 5.5% were diluted to a range of total solids content (between 5 and 1%), both samples decreased in viscosity with total solids. To observe the effect of the serum on tomato puree viscosity, the pellet fraction containing the particles was spun out of the tomato purees by centrifugation so that the particle fraction and the serum could be assessed separately. On further investigation, the viscosity of puree was shown to be affected only to a small degree by the viscosity of the serum. The fact that stirred viscosity was independent of whether the suspension medium constituted of sucrose solution or serum phase showed that little contribution to the viscosity is coming from the soluble hydrocolloids in the tomato sera.

Most dominant for viscosity was the particle fraction. The particle fraction was affected by the maturity of the fruit which in turn was associated with changes in fruit texture, particle size and size distribution of the puree. The particles did not behave as rigid spheres, as shown by the large departure from the corresponding Krieger Dougherty fit (Krieger and Dougherty 1959), ripened fruit deviating the furthest. This suggested the more ripened fruit has softer (less rigid) particles, which was shown to have softer tissue before the pureeing process. This may also have made the tissue more susceptible to rupture into smaller particles during pureeing. Quantitative data for size distribution measured by laser light scattering show that as the tomato ripened from green to red, particles were smaller; the mean diameters for the particles in the four puree samples were 893 μ m (mature green), 542 μ m (breaker), 377 μ m (pink), and 262 μ m (red ripe) and of a broader size distribution.

Pureed mature green (unripe) fruit were shown to offer more potential as an effective natural viscosifier for foods such as soups and sauces, but they may not meet requirements of taste or colour. The last part of this research (chapter 7) therefore focused on tomatoes with variations in their genes rather than ripeness, harvested at breaker + 7 days. Tomatoes at the same stage of ripeness from the *Solanum pennellii* tomato introgression lines (ILs) were grown and harvested from the glass houses at the University of Nottingham. A screening process was developed to compare the viscosity of small samples of tomato puree from 55 of the ILs, 164 fruits in total; the remaining 21 lines did not produce quantities of fruit sufficient for analysis. The samples were diluted with water to the same total solids of 3.5%. The mean stirred viscosity value for all the lines measured was 132 ± 45 cP. The viscosity was calculated from typically three replicate measurements on three individual fruits per line. Although the particle volume and weight fraction of the puree and the initial total solids content had a strong influence on stirred viscosity, it was shown that the variation between individual fruit was greater than that between the different ILs. Previous texture studies of the IL lines showed marked variations yet there was no clear relationship between the puree viscosities and the expected texture of the whole fruits.

ACKNOWLEDGEMENTS

I would like to acknowledge the BBSRC for funding this research. The project was supervised by Professor Sandra Hill. In addition I would like to acknowledge Dr Tim Foster and Dr Bettina Wolf for their supervision and guidance in the early stages of this research. Thank you to Evesham Vale Growers for donating the commercial tomatoes by request of Professor Greg Tucker.

Many, many thanks to Professor Sandra Hill for her faith in me and for her help and guidance throughout, I will remember it always. I also appreciate the advice and encouragement of Professor Graham Seymour. I acknowledge the use of data from experimental work done by Dr Mervin Poole and co-workers in 2006 on colour and texture analysis of tomatoes, detailed in chapter 7. Thank you to Anne-Laure Boue for her assistance and hard work with preparing and analysing the purees from the Introgression lines.

Many people have assisted me with tasks associated with growing, tagging and harvesting of tomatoes at the University of Nottingham. I appreciated the help given by Dr Piotr Jasionowicz, Rebecca Smith, Dr Natalie Chapman, Val Street, Gill West, Dr Bee Lyn Chew, Dr Mervin Poole and the glass house technicians. I would like to thank the members of the Food Science Department for all their help with the time-consuming tasks of washing and de-seeding tomatoes.

Thank you to Dr Rob Linforth, Dr Natalie Chapman and May Ng for their help with statistical analysis. Thank you to Charles Abson for his assistance with spread sheets.

And last but by no means least, thank you to my dear husband and family.

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CHAPTER 1: INTRODUCTION

1 Introduction

The food industry has many requirements for functional ingredients, for thickening and stabilising products. The potential to meet this demand exists in nature, in fruit and vegetables and the fibrous material structures that give them form and rigidity. Once they have been broken down by pureeing, they can be added to processed foods to improve the rheological properties, as shown in the patent by Belmar, Tamai and Thompson (1999). Advantages arise from using minimally processed plant based materials as they are relatively high in fibre and low in fat, and can be listed as natural ingredients on packaging. The UK population are reported to consume less than the recommended daily intake of 18g of Non-Starch Polysaccharides (dietary fibre) per day, (Department of Health, 1991 and Henderson, Gregory and Irving, 2003). Including more fruit and vegetables in the form of thickeners therefore, would be one way of increasing the fibre content in the diet.

Fruit and vegetables contain a combination of water soluble and insoluble material, forming the main structural element of cells and tissues. The cell wall is a major source of these materials. When fruit and vegetables are comminuted they form a thickened paste or puree, caused in part by the interaction of the cell fragments and cell wall materials in solution. The viscosity and other rheological properties depend partly on the morphology of the particles, composition and interactions of the polymers, as well as the processing conditions used to make the puree (Bayod,

Willers and Tornberg, 2008; Errington, Tucker and Mitchell, 1998, Sánchez et al. 2002, Sesmero et al. 2009, Bengtsson and Tornberg 2011, Lopez-Sanchez et al. 2011, Day et al. 2010).

Tomatoes are an important crop worldwide. For example, the United Kingdom imported over 385,000 tonnes of tomatoes in 2010 with a value of over 600 million US dollars 1,235 000 tonnes imported in 2004 (FAOSTAT, 2013). The tomato has also been identified as a suitable model to investigate the effect of the soluble and insoluble materials on rheology. Much research has been carried out previously to understand the effect of cell wall composition on the rheology of the puree, particularly with reference to the changes that occur during the ripening of the fruit and the genetics that affect the cell wall. This is because tomato puree is used extensively in the food industry, and tomato plants have a relatively small genome, short generation period, well characterised mutants and are comparatively easy to work with in genetic manipulation techniques. It is likely that future research in this area will be assisted by the recent completion and publishing of the tomato genome by The International Tomato Genome Sequencing Project (Sato et al. 2012).

Generally only ripe tomatoes are used in food applications. However, tomatoes at different stages of ripeness were investigated in this study because the ripening process is known to affect viscoelastic properties of the puree. For example, cell wall degrading enzymes such as PME (pectin esterase) and PG (polygalacturonase)

modify the structure of pectin in the cell wall during the ripening process (Errington, Tucker and Mitchell 1998). In fact, the fruit cell wall is subjected to a range of enzymes during ripening, which in part soften the flesh (Brummell 2006). This is associated with the dissolution of the middle lamella and structural changes in the networks of cellulose, cross-linking glycans (hemicelluloses), and pectins (Brummell and Harpster 2001). The changes in the cell wall have been relatively well characterised (Seymour et al., 1990), therefore tomatoes of varying maturity make an ideal test material for this study to measure the overall effects of these changes on rheology.

The interdisciplinary position of the research presented in this thesis makes use of a link between two departments at the University of Nottingham; Food Sciences where rheological and structural properties can be investigated, and Plant Sciences where the tomatoes with well characterised genetics can be grown.

1.1 OVERALL OBJECTIVE

The overall objective of the research presented in this thesis was to understand the relationship between tomato tissue and the possible impact of the materials (soluble and insoluble) formed on pureeing on the rheology. Economic implications are that the higher the viscosifying effect of the plant dispersion, the less material has to be added to create the required viscosity of the product. The uniqueness of the research presented in this report is the systematic investigation of the insoluble

and soluble material and how it affected the rheology in tomatoes with known differences in ripeness and texture.

1.1.1 Outline of the thesis

To achieve these overall objectives, the current status and understanding of the effect of ripening, genetics and processing of tomatoes has been undertaken and presented in the literature review. In section 2.6, at the end of the literature review, the specific aims, objectives and hypotheses of the thesis will be stated. In chapter 3 the materials and methods will be presented, and then four research chapters;

- Chapter 4 -The relationship between the texture of the intact fruit and colour of the ripening tomato
- Chapter 5 -Method development for measuring the rheology of tomato puree
- Chapter 6 -Ripeness related changes in the soluble and insoluble components of tomato puree and the effect on rheology
- Chapter 7 -Harvesting and measuring tomatoes from a set of *S. pennellii* introgression lines (a series of cultivated tomato species containing the genetic material derived from a wild variety of tomato species) at the same ripeness (breaker + 7 days), to determine if any have notable increased viscosity.

CHAPTER 2: LITERATURE REVIEW

2 INTRODUCTION

Figure 2-1 encapsulates the basis of this thesis, in the understanding of how the rheology of the whole fruit and the tissue can affect the rheology once it has been processed. Fruits and vegetables form a suspension of particles within a serum prepared by breaking down the fruit tissue during a pureeing process. The hierarchical nature of structuring is shown; from the macro to the molecular. The largest structural entity, the whole fruit (centimetres) is made up of tissue, formed from individual cells joined together (in the size range of millimetres). On pureeing the flesh of the fruit is broken down into particles. These are insoluble and of varying sizes; whole cells, disintegrated cells and cell wall material. The mechanical force ruptures some of the cells, causing damage and releasing a serum of soluble components such as pectin, simple sugars, salts and acids (Bayod et al. 2007, Yoo and Rao 1994, Gancedo and Luh 1986). These are the molecular components and the smallest structures that will be studied. The viscoelastic properties of the insoluble solids can be measured separately from the serum by centrifuging the puree, Figure 2-2.

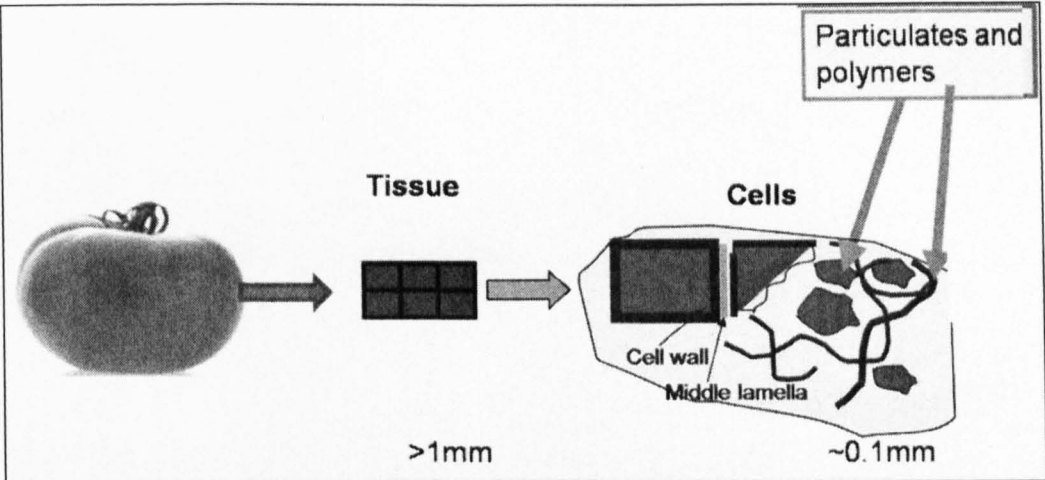


Figure 2-1 Schematic representation of the effect of processing on tomato tissue and the resultant suspension of particles and serum (polymer and simple sugars and acids in solution)

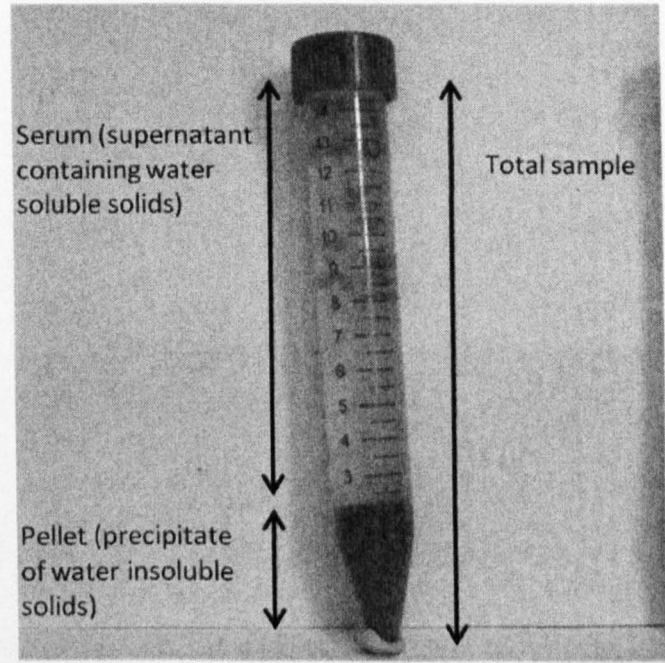


Figure 2-2 Separation by centrifugation of tomato puree into water soluble (serum) and water insoluble particles (pellet). The pellet also contains some soluble solids.

This is an innovative approach compared to looking at the entire pureed or pasted material as a whole.

2.1 TOMATO PUREE AND PASTE

Terms such as pastes and purees depend on the concentration of the materials from tomato. Commercial products are typically produced using the following steps

using ripe tomatoes, Figure 2-3. Tomato harvests are graded on colour, soluble solids and percentage of defects, then washed to remove debris, dirt mould and insects, and then sorted to remove unripe and rotten tomatoes and leaves etc.

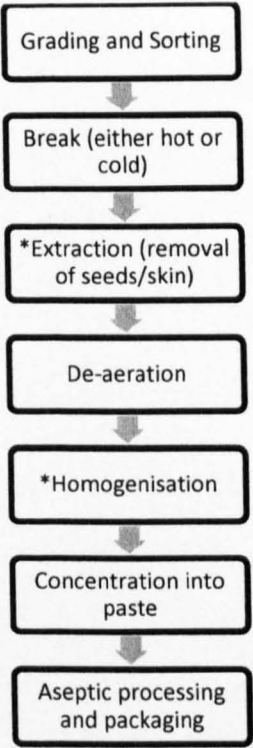


Figure 2-3 Main steps in manufacture of tomato paste. * indicates processes that affect particle size. Adapted from (Barringer 2004).

Either a hot or cold break process can be used in the next step. This involves chopping the tomatoes (often under vacuum to minimise degradation of ascorbic acid) whilst heating. In the more common hot break, the tomatoes are chopped and rapidly heated to at least 82°C to inactivate pectin degrading enzymes, especially polygalacturonase and pectin methyl esterase. During the equivalent cold break step, tomatoes are chopped and gently heated to 40-60°C to promote the enzyme activity and increase yield. Cold break is generally considered to have better colour and flavour, but has lower viscosity due to the enzyme activity. Cold break puree is more often used in tomato juice and juice based drinks.

To remove skin and seeds, the tomato is then put through an extractor, pulper or finisher. Finishers range from 0.7mm to 4mm. Extraction of the puree can be done with a screw type or paddle type extractor. The tomato puree is pushed through a screen which affects the particle size. De-aeration removes dissolved air, and then homogenisation is done to increase viscosity and reduce serum separation. This process forms tomato puree, and is typically between 7 and 24% natural total soluble solids (NTSS). If concentration of the product is required to make tomato paste, it is done in forced circulation, multiple effect vacuum evaporators. A typical four effect evaporator increases the temperature in successive steps from typically 50-80°C. Vacuum is used so that a more gentle heat can be applied, minimising colour and flavour loss. The paste is concentrated to generally 26 to 31% NTSS. Finally, the tomato paste or puree is pasteurised, e.g. 109°C for 2 minutes, and packed into containers (Barringer 2004).

During processing, care must be taken to ensure a high quality product is made in terms of colour, taste and rheological properties. The economic implications are the higher the viscosity of the tomato paste/puree, the less needs to be added to reach the desired final product consistency.

Parameters important to viscosity have been studied in depth previously, and some of the factors important to viscosity have been reported as follows. The viscosity of ripe tomato products is increased with total solids (Rao, Bourne and Cooley 1981),

and pulp content, (equivalent to the pelletable material referred to in Figure 2-2).

The pulp content (particle fraction) has a larger effect on the viscosity of the puree than the serum viscosity (Tanglertpaibul and Rao 1987). Serum viscosity is Newtonian and increases with the soluble solid content, as measured by Brix, shown in Figure 2-4. Serum viscosity is also affected by the mesh size in the finishing step (Yoo and Rao 1995). The amount of pectic substances in the serum of tomato puree at 5.6% Brix is in the region of 0.16% (Tanglertpaibul and Rao 1987). Other important factors include the screen size in the finishing step, (Yoo and Rao 1995), and homogenisation (Lopez-Sanchez et al. 2011). The choice of mesh size in the finishing step and the severity of the homogenisation affect particle size and particle size distribution, which in turn affect the volume fraction of the particles.

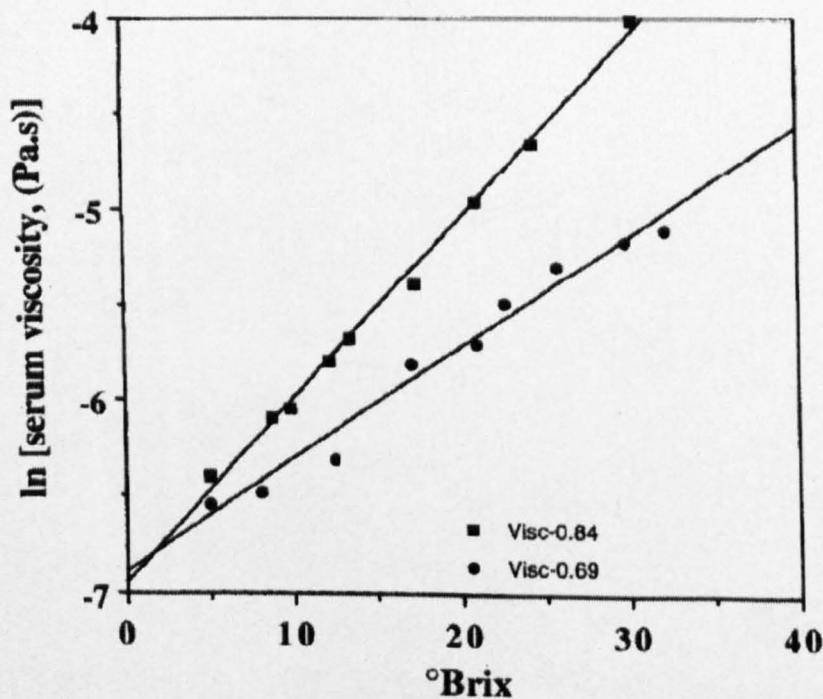


Figure 2-4 Serum viscosity as a function of concentration of ripe tomato paste (concentrate) made using 0.69 and 0.84 mm finisher screens (Yoo and Rao 1995).

Processing is not the only factor affecting the rheological properties of tomato products; microstructures are the result of the processing conditions and the properties of the starting material – the tomato fruit.

2.2 USE OF TOMATO AS A MODEL FOR INVESTIGATING THE CONTRIBUTION OF INSOLUBLE AND SOLUBLE MATERIAL TO TOMATO RHEOLOGY

Botanically, the tomato is a fleshy berry; the anatomical features of a typical fruit are shown in Figure 2-5, with the waterproof outer cuticle (skin), seeds, pericarp (the ovary wall and the fleshy part of the fruit) and gelatinous parenchyma surrounding the seeds. The inner and outer pericarp tissues are separated by vascular tissue. The inner and outer pericarp together are known as the mesocarp, or more generally, the pericarp.

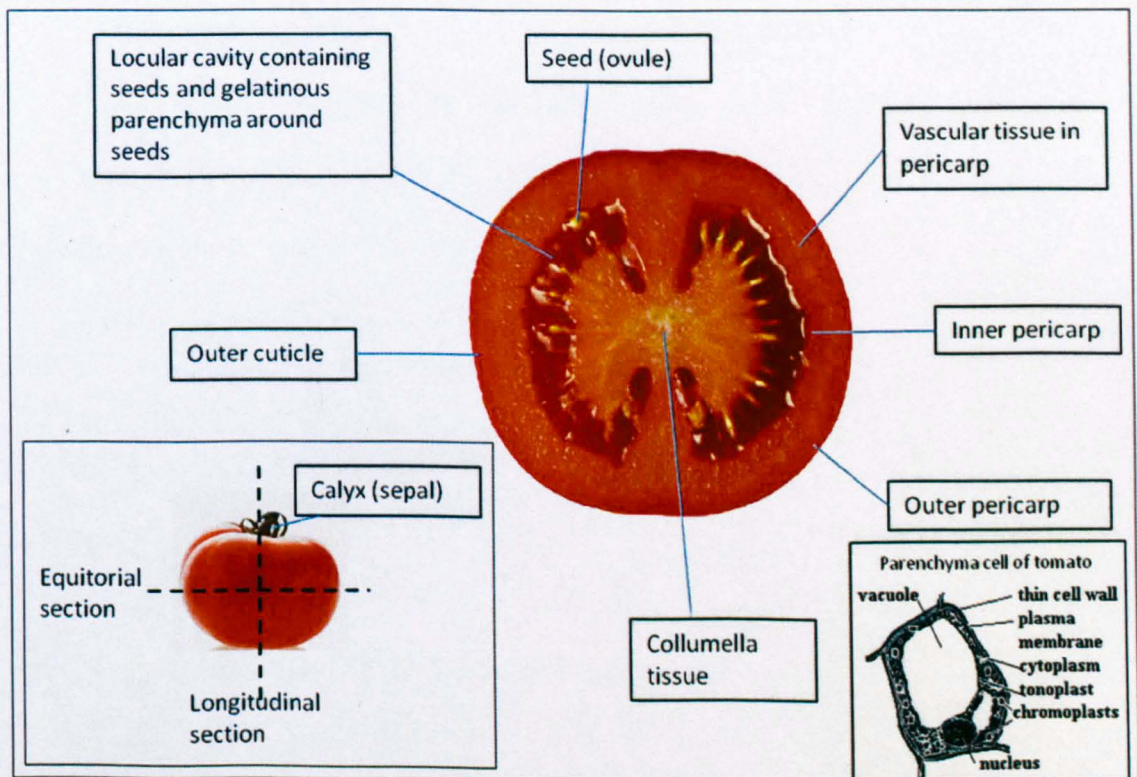


Figure 2-5 The anatomy of a tomato fruit.

The smaller diagram in Figure 2-5 shows the components of a typical tomato parenchyma cell of the mesocarp. It shows the cell wall forming a permeable outer layer of the cell, followed by a selectively permeable plasma membrane. Jointly they separate the contents of the cell from the environment. The apoplast (not shown) is the area between plant cells, an intercellular space that surrounds every plant cell, in which metabolic and physiological processes relating to cell wall biosynthesis, nutrient transport, and stress responses occur. The vacuole, shown on the diagram above, typically occupies 30-80% of the plant cell, and is separated from the cytoplasm by the tonoplast. The main functions of the vacuole are storage and to maintain turgor pressure on the cell wall. Cell turgor pressure arises because of the sugars and salts in the vacuolar fluid causing an osmotic pressure gradient across the plasma membrane pushing against the cell wall. Turgor is one of the parameters that affect texture of the whole fruit and tissues.

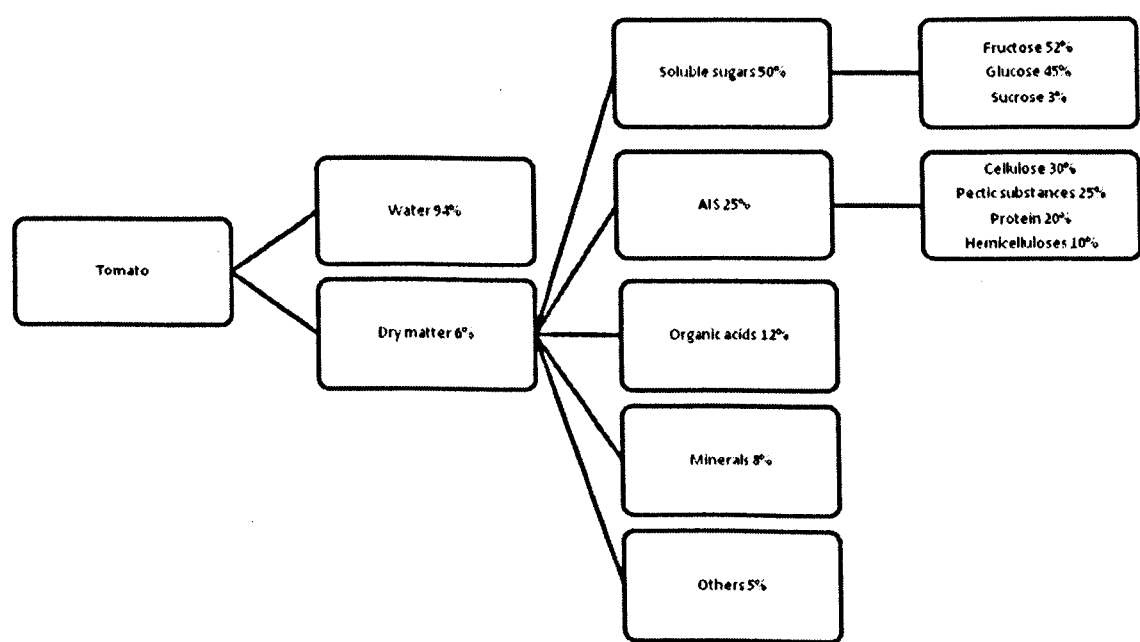


Figure 2-6 Typical composition of tomato fruit, as summarised by (Heutink 1986).

The typical composition of ripe tomatoes has been summarised in Figure 2-6. The dry matter (6% of the total mass) is made of soluble and insoluble material. 50% of the dry matter is soluble sugars consisting of mainly fructose, glucose and smaller amounts of sucrose. Other soluble material includes the organic acids at 12% of the total dry weight. These contribute to the characteristic sweetness and acidity of tomato flavour. A proportion of the pectin is water soluble. The fibrous, insoluble material can be defined in several ways, and the exact composition is dependent on the method used to determine it. In the figure above, it has been determined as the AIS (alcohol insoluble solids). The AIS content of tomatoes has been shown to be highly correlated to Brookfield viscosity, (Janoria and Rhodes 1974). The AIS is often referred to as the cell wall material as it mainly consists of cellulose, pectins and hemicelluloses. As will be discussed in the next section, these form the complex, dynamic structure of the cell wall.

2.3 THE CELL WALL

As early as the 1950s it had been recognised that the cell walls, although only a small proportion of the solids in tomato products, have a significant effect on the viscosity of tomato puree (Whittenberger and Nutting 1958). Cell walls have many components and functions, but only the main points about the structural polysaccharides will be reviewed here. The cell walls are composite biopolymer structures of cellulose, hemicelluloses and pectins, Figure 2-7, with other components such as structural proteins. Each of these materials serve different purposes to give the structural strength, rigidity, flexibility and porosity for

individual cells to function and to join together to form tissues. The cell walls are the main structural element in fruits and vegetables. The structure of the cell wall is complex in terms of the individual components and their interactions (Carpita and Gibeaut 1993, Brett and Waldron 1990), and there is still some debate as to how the three main components interact. Figure 2-7 shows the cellulose/hemicellulose network as the load bearing part of the cell wall that interacts with the pectin network that adds flexibility.

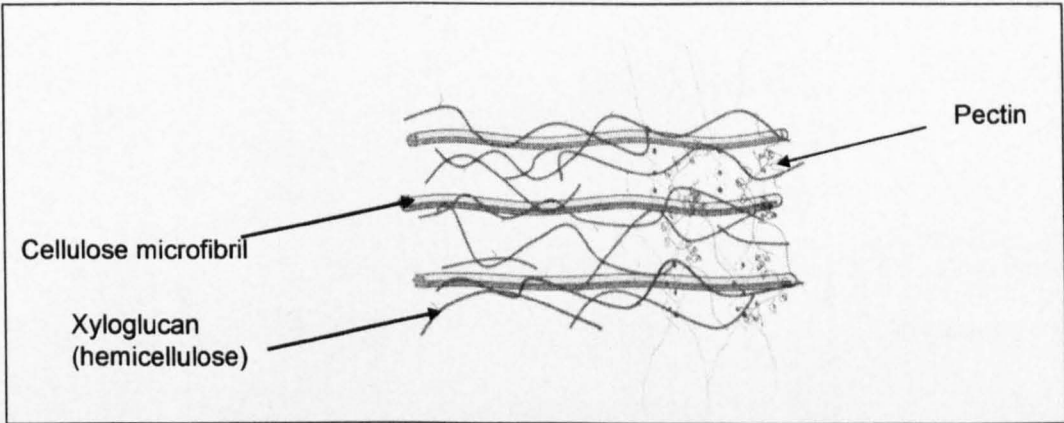


Figure 2-7 The primary plant cell wall (flowering plants) showing the cellulose/hemicelluloses network, separate from the pectic network. Adapted from (Carpita and Gibeaut 1993)

Researchers have used a range of approaches to elucidate the structures and functions of the cell wall polysaccharides. Extraction of crude cell wall material can be done with other solvents such as acetone and ethanol, SDS (sodium dodecyl sulphate) or water to yield insoluble solids. These insoluble solids can be sub-fractionated, or sequentially extracted to isolate cell wall components according to their solubility in a range of solutions, Table 2-1. The cell wall material is added to the first solution, (for example water), and is filtered. The filtrate contains the water soluble component of the cell wall, and can be dried to a powder. The material that was insoluble in the first solution (water, in this case) is then mixed

with a second solution, this time a chelating agent such as CDTA (cyclo-hexane-trans-1,2-diaminetetra-acetate), and the process of filtration and separation done again to obtain the CDTA soluble and insoluble portions, and so on. The materials that solubilise in each solution can be analysed to gain information on the structure or composition or physical properties, as was done on tomatoes (Seymour et al. 1990).

Table 2-1 Typical solvents and the cell wall components solubilised in the sequential extraction of cell wall material Adapted from (Izydorczyk 2005)

Solvent	Material solubilised
Water	Water soluble pectins
Chelating agent (e.g. CDTA/ EDTA)	Remove calcium from the cell wall and solubilise the ionically bound pectin from the cell wall and middle lamella
Sodium carbonate	Covalently bound pectins, released from the cell wall by de-esterification. The extracted pectin is of relatively low molecular weight
Weak alkali (e.g. 1M KOH)	Solubilisation of weakly bound hemicelluloses; the loosely bound matrix glycans such as glucomannan
Strong alkali (e.g. 4M KOH)	Solubilisation of other hemicelluloses; the more strongly bound matrix glycans
Insoluble residue	Mainly cellulose, with pectin, and to a lesser extent glycans.

Monoclonal antibodies have been widely used to analyse the pectin, such as JIM5, JIM7, LM7, PAM1 and F2, and hemicelluloses within cell walls, with the advantage of visualising the location of specific structures in the context of intact cell wall architecture. For a review on the generation and use of antibodies in cell wall structural analysis, see Willats and Knox (2003). Analysis of cell wall components *in situ* in relation to intact cell wall architecture is important because it has given

insight of the localised effect of enzymes during the ripening process on the middle lamella, for example, and can show where specific structures exist within the composite structure of the cell wall. Much work has been done separately in the analysis of the polymers (e.g. pectin), and on purees made by breaking down fruit tissues, for example with genetically modified tomatoes.

As part of this current work, the size level above the cell wall in the hierarchy of structures is studied, by investigating the rheology of the particle material that contains the majority of the cell wall material. But first it is important to understand the structures within the cell wall material, the cellulose, hemicelluloses and pectins.

2.3.1 Cellulose

Cellulose is a polysaccharide of (1-4) β -linked D-glucose molecules. The chains can be up to 4 microns in length and occur in bundles, or microfibrils, as shown in onion cell wall, Figure 2-8. Each microfibril contains approximately 40-80 chains of cellulose (Cosgrove, 2005) and is 10-25 nanometres in diameter. Cellulose microfibrils twist together to form fine threads that may coil around one another like strands in a cable (Raven, Evert and Eichhorn, 1999).

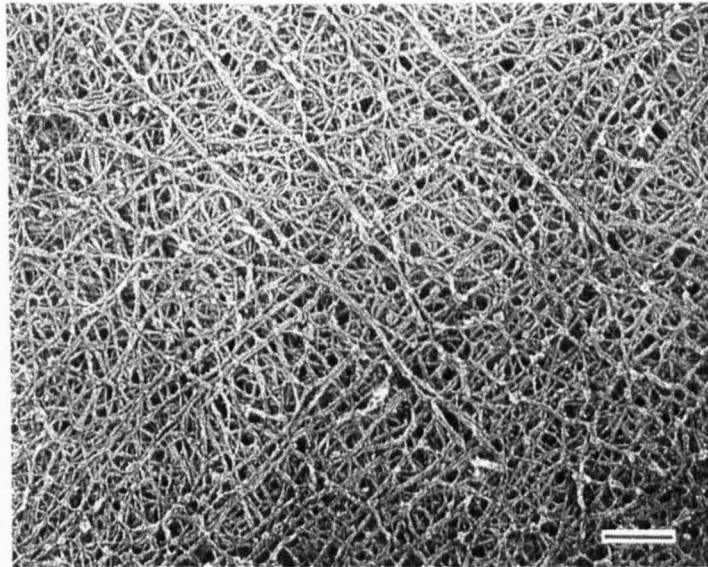


Figure 2-8 Electron micrograph of cellulose microfibrils in onion cell wall. Scale bar 200nm (McCann, Wells and Roberts, 1990).

Cellulose is a relatively rigid, linear molecule because of the restricted rotation around the β 1-4 linkages between each glucose molecule. Additional strength is created through the intermolecular hydrogen bonding that occurs between the hydroxyl groups of adjacent cellulose molecules. The microfibrils occur around the whole of each cell and are the main architectural unit of the plant cell wall. Due to the extensive hydrogen bonding, cellulose has a semi-crystalline structure. Its high molecular weight means that cellulose is insoluble in water. Humans do not produce cellulases to break down this material, so it is also classed as a source of insoluble fibre in the diet.

2.3.2 Hemicelluloses

The cellulose-hemicellulose network is considered to be the major load bearing structure in the primary wall. Hemicelluloses are associated with and bridge the span between the cellulose microfibrils. McCann, Wells and Roberts (1990) have

shown this association in onion cells. Hemicellulosic polysaccharides can be defined as cell wall polymers that are solubilised in weak and strong alkali solution, such as in the sequential extraction shown in Table 2-1. They include xylan, glucuronoxylan, arabinoxylan, mannan, glucomannan and galactoglucomannan; they all generally consist of a cellulosic backbone and differ in their side chains. The difference between cellulose and xyloglucan, for example is that about 75% of the glycosyl backbone residues of xyloglucan are branched bearing α -D-xylose residues at the O6 position and many of these xylose residues have glucose substituents at the O2 position. The hemicelluloses constitute approximately 10%, perhaps 20-30% (Sakurai and Nevins 1993), of the AIS. The compositions of the hemicelluloses are dependent on the species of the plant and type of cell. For example, the main hemicellulose found in tomatoes is a xyloglucomannan (xyloglucan), (Seymour et al. 1990).

2.3.3 Pectins in the cell wall and middle lamella

Approximately 30% of tomato cell walls are pectin (Carpita and Gibeau 1993). The changes in the pectin structures have been shown to be a contributing factor in the softening of ripening fruits, as described in section 2.3.4. Structurally, the pectins are a complex group consisting of covalently linked polymers that consist mainly of galacturonic acid, rhamnose, arabinose and galactose (Brett and Waldron 1990). The synonymous element of all these polymers is that they contain homogalacturon, 1-4 linked α -D-galacturonic acid residues, represented in Figure 2-9. Rhamnogalacturonan I (RGI) has a repeating disaccharide unit of 1-4-linked α -D-

galacturonic acid and 1-2 α -L-linked rhamnose. Glycan chains such as arabinan and glycan are linked to the rhamnose sugars. Rhamnogalacturonan II (RGII) has a backbone of homogalacturon and complex side chains linked to the galacturonic residues. Alternative arrangements of the primary structures of pectin have been proposed, as shown in B of Figure 2-9.

As shown in Figure 2-7 and in research done by McCann et al. (1990), the covalently linked (sodium bicarbonate –extractable) pectins form a network separate from the cellulose-hemicelluloses, so that the cell wall structure is made up of two distinct networks. However the polymers in the cell wall are closely linked in many ways, as it has also been shown by research such as by Foster et al (1996) that some pectic material is also strongly associated with the cellulosic part of the cell wall. The finer details of the structures and interactions of the cell wall polymers are still being researched.

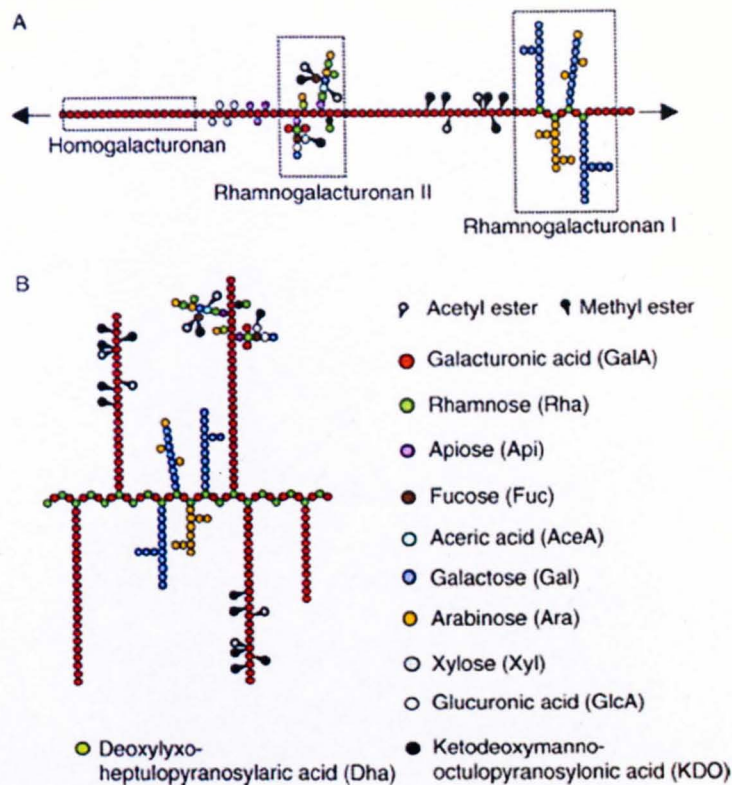


Figure 2-9 The basic structure of pectin.

[Schematic representations of the conventional (A) and more recently proposed alternative (B) structures of pectin. The polymers shown here are intended to illustrate some of the major domains found in most pectins rather than definitive structures. Diagram from (Willats, Knox and Mikkelsen 2006)]

However, it is known that the middle lamella, depicted in Figure 2-10, is a pectin rich area of the cell wall that is devoid of cellulose microfibrils. It acts as the adhesive between adjoining cells, thus forming plant tissue. Homogalacturonan chains that form extensive cross linkages condense to form rigid structures with little mobility and are found most at the tricellular junctions (Jarvis and McCann, 2000).

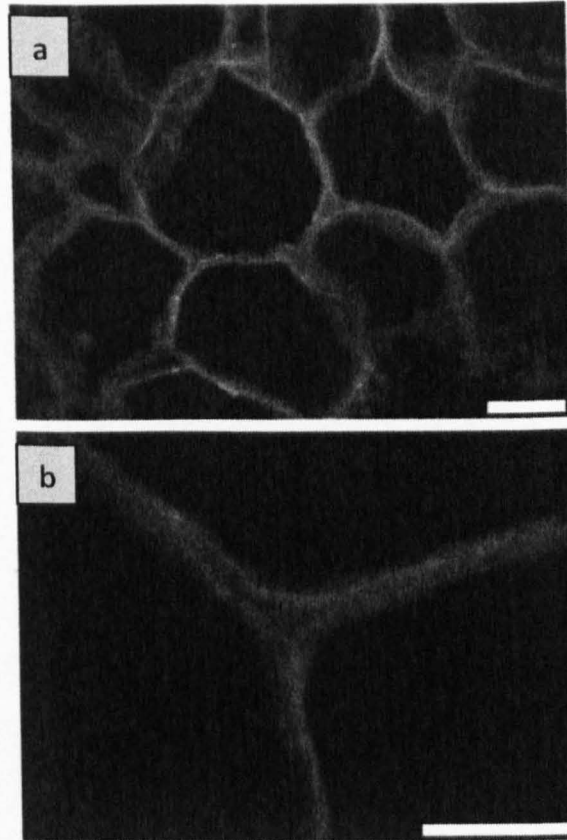


Figure 2-10 Location of the middle lamella in adjoining tomato cells.

[As shown by images of PME in tomato pericarp using immunofluorescence detection and MA-ASP-25F7 as the detecting antibody, (a) in the cell wall (b) of a tricellular junction. Scale bar (a) 200 μ m, (b) 100 μ m. Adapted from (Vandevenne et al. 2011)]

2.3.4 Changes to pectins and hemicelluloses during ripening

Many enzymes have been reported to increase in activity during the ripening of fruit, as reviewed in Brummell and Harpster (2001). Enzymes that cause reduction of molecular weight of pectins during ripening are polygalacturonase (PG) and pectin lyases (PL); the enzyme that reduces the degree of methyl esterification is pectin methyl esterase (PME). Their sites of action on polygalacturonic acid (homogalacturonan) are represented in Figure 2-11. PG hydrolyses the galacturonan backbone of pectic polysaccharides whereas pectin lyases catalyse the cleavage of unesterified galacturonosyl linkages via a β -elimination reaction. The

methyl group is located at the C6 position of a galacturonic acid residue and is the site of activity of PME, where it removes the methyl group. The degree of pectic esterification decreases from 90% in immature green fruit to 30% by the red-ripe stage of ripening (Koch and Nevins 1989). The action of PME (there is more than one form of PME) on the methylesterified pectin show a gradual increase from the immature green stage right through to the pink stage then slowly declines as ripening progresses further (Errington et al. 1998). Since the pectic polymers begin to acquire solubility only after PG has become active, it is believed that this enzyme is involved in the breakdown of some of the insoluble complex polysaccharides by reducing the length of the chains cross-linked by calcium.

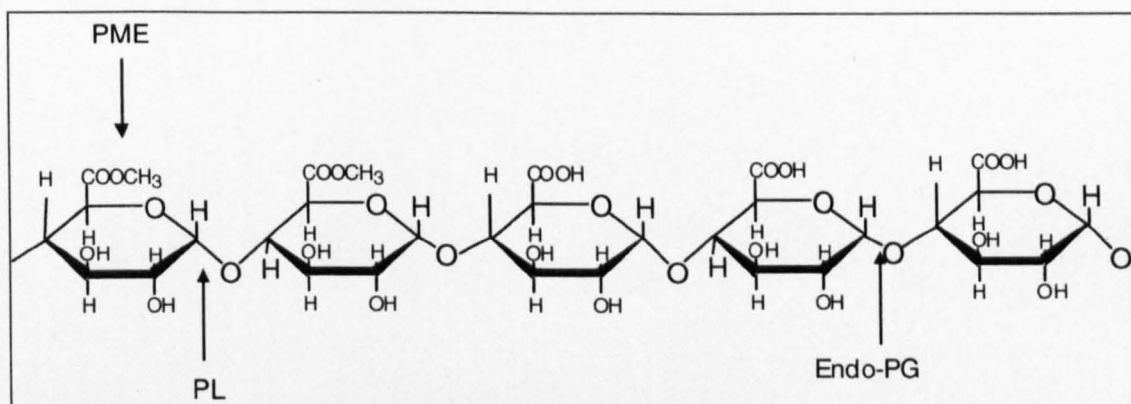


Figure 2-11 Pectic degradation enzymes and their sites of action on homogalacturonan.

[PME – pectin methyl esterase, PL – pectin lyase and Endo-PG –polygalacturonase. PL and PG reduce molecular weight and PME reduces the degree of methyl esterification during the ripening of tomatoes.]

During ripening, the cumulative effect of enzymic action on the pectins and hemicelluloses is a progressive modification of the cell wall architecture, causing softening of the fruit texture. A slightly softened texture denotes to the consumer that the fruit is ripe and will be pleasant to eat. An over-soft fruit has an unappealing texture for the fruit to be eaten fresh, and gives a poor product when pureed or tinned. As shown in Figure 2-12, the CDTA-soluble pectins (measured as uronic acid), and the matrix glycans (measured as neutral sugars) show a decrease

in molecular weight in the red ripe tomato compared to the unripe mature green. A reduction in the molecular weights of the hemicelluloses has been reported to occur during the ripening of tomatoes (Huber 1983), but the effect of the changes in hemicellulosic structures on the softening of fruit is less clear. The loss of neutral sugar side chains from pectin is also an important change occurring during ripening and affects the pectic network in the cell wall.

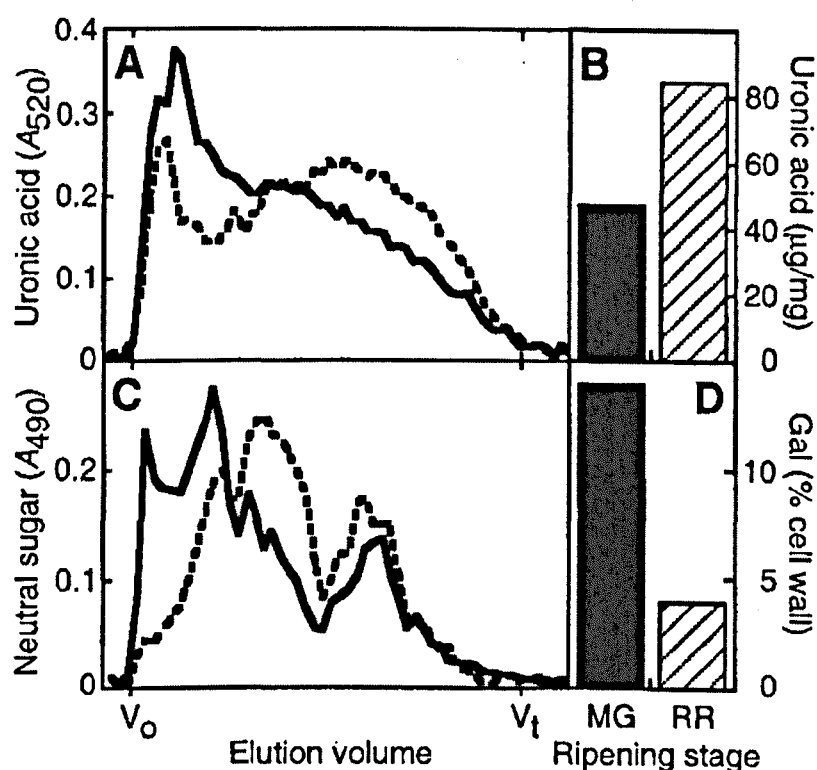


Figure 2-12 Changes to pectin and matrix glucans (hemicelluloses) during the ripening of tomato fruit. From the review by Brummell and Harpster (2001).

[Solid lines and black bars denote mature green (MG) fruit; broken lines and hatched bars, denote red ripe (RR).

A. Depolymerisation of chelator (CDTA)-soluble pectin. Molecular weight profile of CDTA-soluble polyuronide after size exclusion chromatography on Sepharose CL-2B (V_o = 20 MDa, V_t = 100 kDa).

B. Amount of chelator-soluble pectin. Expressed as µg of CDTA-soluble uronic acid per mg ethanol-insoluble cell wall material.

C. Depolymerisation of matrix glycans tightly bound to cellulose. Molecular weight profile of 24% KOH-soluble matrix glycan after size exclusion chromatography on Sepharose CL-6B (V_o = 1 MDa, V_t = 10 kDa).

D. Galactose content of the cell wall. Non-cellulosic neutral sugar composition of crude cell walls was determined by gas chromatography and expressed as a percentage of cell wall.]

The highly methyl esterified (HM) form of pectin in unripe fruit is de-esterified by PME as the fruit ripens, resulting in low methoxy (LM) pectin. The unbranched, de-esterified chains of LM pectin can aggregate through calcium binding to form the cell junction zones that create a gel-like structure, represented in Figure 2-13. The negatively charged carboxylic acid groups of pectins cause them to bind with calcium (or other divalent cations) by association of two separate chains. The resultant structure is known as the 'egg box' model of gelation. In plants, the ionic

bonds of the calcium between HG polymers play an important role in maintaining cell wall integrity and cell–cell cohesion.

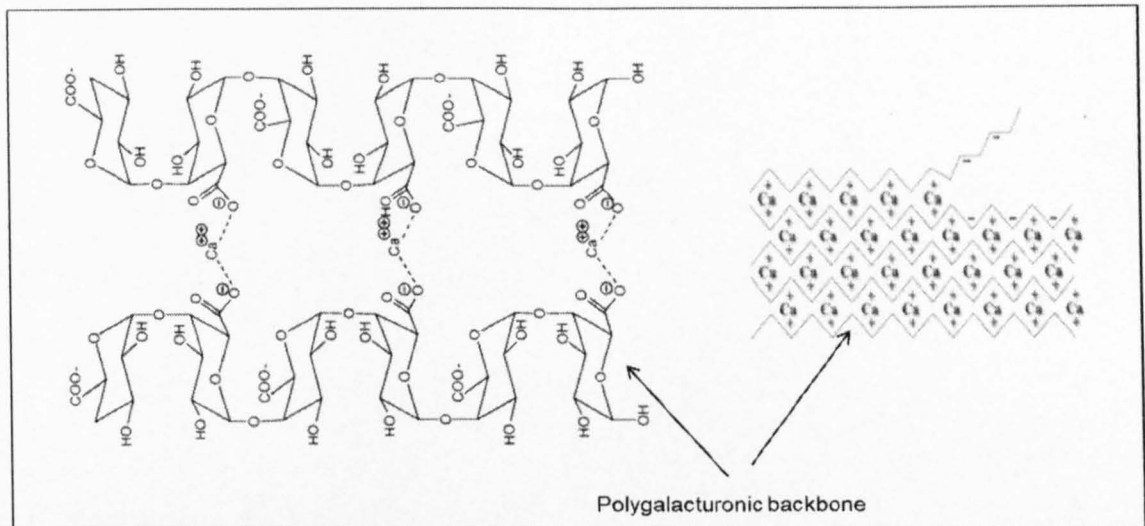


Figure 2-13 Pectin association.

[On the left; a depiction of the association of two separate chains of low methoxy pectin (polygalacturonic backbone) with Ca^{2+} ions at the negatively charged carboxylic acid groups, and on the right, the Egg-Box model depicting many chains of pectins associating with calcium ions in this manner. Adapted from (Grant et al. 1973).]

These changes to the primary structures (i.e. to the hemicelluloses and the pectins) during ripening cause the cell wall composite structure to become more hydrated. As the pectin structures are modified, changes in the pectin network in the cell wall and in the middle lamella ensue. This is considered one of the main factors influencing the ease with which the cells can be separated from each other, or broken up during the production of tomato purees. Figure 2-14 shows the relationship between polygalacturonase (PG) activity in tomato compared to the amount of water soluble pectin at different stages of ripeness (Gross and Wallner 1979), which could suggest a change in the composition of the serum of pureed tomatoes may occur.

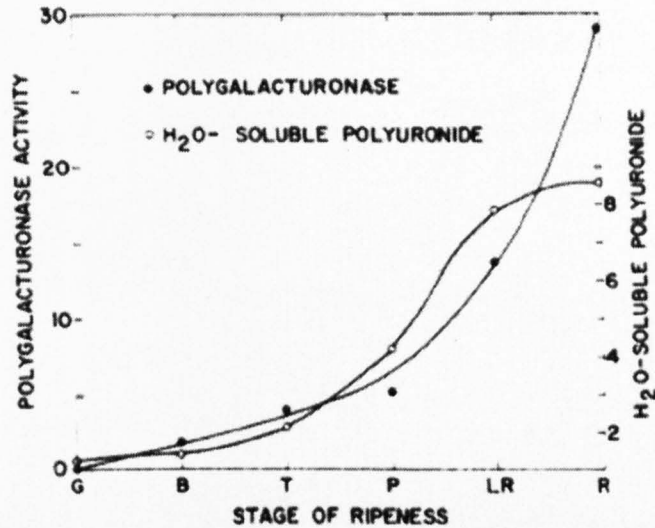


Figure 2-14 Polygalacturonase activity in citrate extracts and water-soluble polyuronide of isolated cell walls from tomato (cv. Heinz 1370) fruits at different stages of ripeness. (Gross and Wallner 1979)

[(G - green, B- breaker, T-turning, P-pink, LR-light red, R-red). Enzyme activity is expressed as tamol product/hr.g fresh wt; water-soluble polyuronide units are mg galacturonic acid/100 mg cell wall.]

Other reasons for the softening and separation of cells have been investigated. For example, the role of the ripening-specific expansin Exp1 (a cell wall structural protein) in fruit softening and cell wall metabolism was investigated by suppression of Exp1 in transgenic tomato plants. Fruit in which Exp1 protein accumulation was suppressed to 3% that of wild-type levels were firmer than controls throughout ripening (Brummell et al 1999). Others have investigated the possibility that pectin solubilisation and cell separation in fruit may be due to organic acids disrupting calcium bridges between pectic polysaccharides (Macdougall, Parker and Selvendran 1995).

2.3.5 Developmental changes during tomato fruit ripening of traditional tomato

The softening of tomatoes during ripening has been quantified with texture measurements on whole fruits, with the approximate ripeness gauged by the

external colour as the fruit changes from green to red. This reduction in firmness was shown to be reflected in the loss of viscosity on ripening for control and transgenic tomatoes (Powell et al. 2003). If the tomato is too ripe and left too long before harvest and processing, a decline of product quality can occur and this deterioration is mainly due to the amount of softening.

Figure 2-15 shows the major developmental changes during tomato fruit development and ripening. This has relevance to the current work because the ripeness of the tomatoes used in experimental work was graded in two ways. The stage of ripeness of the commercial tomatoes was graded on the colour of the skin, and the tomatoes from the *S. pennellii* introgression library were harvested at 7 days post breaker. Ripening involves multiple reactions, starting at anthesis and fertilisation of the flower. From then, fruit growth consists of rapid cell division within the first 7-8 days and then a phase of cell expansion, until approximately day 35 post anthesis. At approximately 40 days post anthesis, the tomato is in the mature green stage, has reached maximum size and has mature seed. It is assumed that the pericarp cells have reached maximum size at this stage, and the cell size remains relatively constant through the ripening stage. At the breaker stage, the green tomato shows the first flush of pink/red colour, and then it changes to orange/pink to red ripe. The first pigment change is a fading of the green colour due to the transformation of chloroplasts into chromoplasts resulting in a decrease in chlorophyll concentration. The initial increase in β -carotene concentration results in the orange/pink pigment. The final red colour of a red ripe fruit is due to the

subsequent high concentrations of lycopene and β -carotene (Grierson and Kader 1986, Fraser et al. 1994). The redness of the fruit remains relatively constant from this stage, but other developmental changes continue resulting in the fruit becoming over ripe and overly soft.

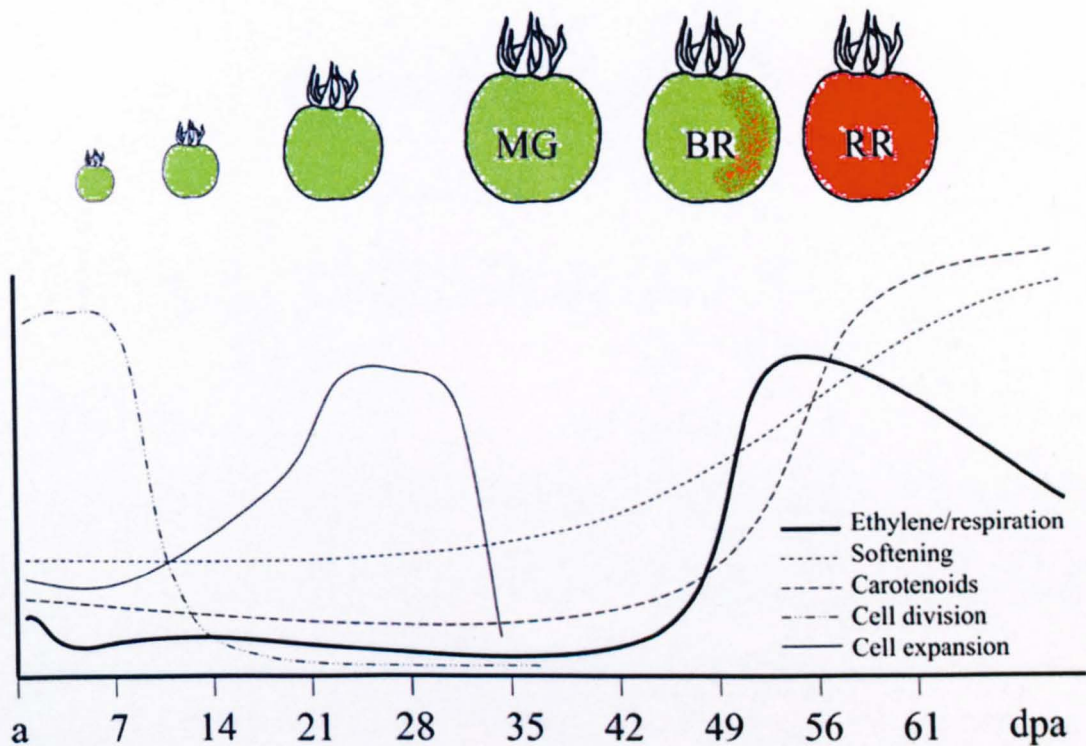


Figure 2-15 Major developmental changes during tomato fruit development and ripening.

[a –anthesis, dpa, days post anthesis. MG – mature green fully expanded unripe fruit with mature seed and occurs at approximately 40 dpa, BR – breaker, first visible carotenoid accumulation, RR – red ripe. Adapted from (Giovannoni 2004).]

The respiration rate and ethylene production increase dramatically at the breaker stage. As with other climacteric species, the release of the plant hormone ethylene (C_2H_4) causes the onset of the major ripening characteristics. This is the plant's signal causing many enzymic reactions resulting in the softening of the fruit, including PME and PG, as discussed.

Cellular turgor pressure, i.e. the hydrostatic pressure of cells, a determining factor in firmness and has been shown to decrease with ripening (Shackel et al. 1991). It is postulated that this loss in turgor is due to two main factors. The first is an accumulation of osmotic solutes in the apoplast and water loss from the fruit meaning there is a smaller difference in the osmotic potential between the cell vacuole and the apoplast. The second is that ripening related softening of the cell wall means that it would have less mechanical strength for the cell contents to push against, thus turgor pressure is reduced. A decline in turgor reduces the expansionary pressure on the cell wall, exerted by the vacuole on the cell membrane, which in turn presses against the cell wall creating the pressure.

2.4 GENETICALLY MODIFIED, MUTANT AND INTROGRESSION LINE TOMATOES

Much of the understanding of ripening-related changes has been approached by use of antisense technologies that have been used to alter gene expression in tomatoes and by characterising tomato ripening mutants. Antisense technology uses a nucleotide sequence that mimics a DNA sequence, but cannot serve as a template for the messenger RNA, thereby disrupting genetic replication. This interrupts the normal cellular processing of the genetic message of a gene, and the end result is to 'silence' or knockout the gene. This allows the research to determine the particular function of a gene. For example a polygalacturonase (PG) antisense (Smith et al. 1988), pectin methyl esterase (PME) antisense (Hall et al. 1993), ripening-specific Expansin Exp1 protein (Brummell et al. 1999) lines have all been studied to determine the effect of silencing these genes during ripening and

have been shown to have increased viscosity in puree. Suppression of PG reduces fruit softening only slightly (but shelf life is extended). Suppression of pectin methylesterase activity had little effect on fruit firmness or ripening characteristics. However, Jimenes-Bermudez et al. (2002) reported that the strawberries from transgenic plants containing an antisense sequence of a strawberry pectate lyase gene were firmer than the control fruit. Sesmero et al. (2009) found that the juice of pectate lyase-silenced strawberries had altered rheological properties (i.e. higher G' and G'') and an increased viscosity than the control fruits. When pureed, the transgenic fruit showed no differences in the soluble solids, pH, or solid volume fraction. Instead, the higher viscosity was attributed to an increased content of large particles in the juice, a slightly higher content of large molecular mass polyuronides, when compared to the control (Sesmero et al. 2009).

Tomato ripening mutants of interest are tomato varieties that have single gene mutants that abolish normal ripening in tomato and include rin, nor and Cnr, ripening inhibitor, non-ripening and colourless-non ripening, respectively. All fail to produce the burst of ethylene typical of non-mutated lines and have fruit which generate puree with altered viscosity characteristics in comparison to wild type.

Puree from genetically modified tomatoes was for sale in the UK in the 1990's with reduced levels of PG. In the USA a reduced PG fresh market tomato, Flavr Savr, had delayed softening so could be left on the vine longer than standard commercial

tomatoes and so had time to develop good flavour characteristics. However, these tomatoes were susceptible to cracking of the skin, and were also uneconomical to produce. In the UK, GM technology began to be unpopular with British consumers.

2.4.1 *S. pennellii* tomato introgression lines

There is a clear need for improved fruit characteristics for tomatoes, for texture and viscosity. Traditional breeding and improvement of tomatoes has been done for many years, but has been hampered in part, by the narrow genetic base of *S. lycopersicum*, reducing the possibility of improvement with this approach. *S. lycopersicum* is the tomato from which standard cultivated tomatoes are derived and it was this type that was used in the experimental work reported in research chapters 4, 5 and 6. However, wild tomato species have proven useful as a way to introduce additional variation into the cultivated background. In research chapter 7 tomatoes of known genetic variation were used. These were the set of *S. pennellii* tomato introgression lines where each line has a single marker defined segment of wild species genome introgressed into the cultivated background creating a genomic library of *S. pennellii* segments in an *S. lycopersicum* background (Eshed and Zamir 1995) and can be used to identify quantitative trait loci or QTL for discrete genetic variation within a population for crop improvement purposes. Once these QTLs have been identified, the desired traits can be transferred to commercial varieties of tomato through introgression breeding. Analysis of the introgression lines therefore, has the potential of developing tomatoes with better processing qualities, over the longer term.

Figure 2-16 can be used to explain the concept of introgression lines and how they can be used to identify of the possible genes (or chromosome segment) responsible/related to increased viscosity, or any other trait one might be interested in. The 12 green (top) and 12 red (bottom) chromosomes represent the genomes of *Solanum pennellii*, a wild green fruited tomato, and *Solanum lycopersicum* CV M82 a cultivated tomato, respectively. The introgression lines (ILs) contain 76 lines which consist of nearly entirely of generic material from the M82 except on one specific section on one of the 12 chromosomes which is the *S. pennellii* introgressed fragment. For example, IL 1-1 is shown in the diagram has the wild type genetic material located on the first part of chromosome one, and the rest of its DNA is from the M82 cultivated tomato. On IL 12-4, the genetic material from *S. pennellii* is located on another section of the chromosomes. In this way, each line contains a single homozygous marker defined *S. pennellii* chromosome segment.

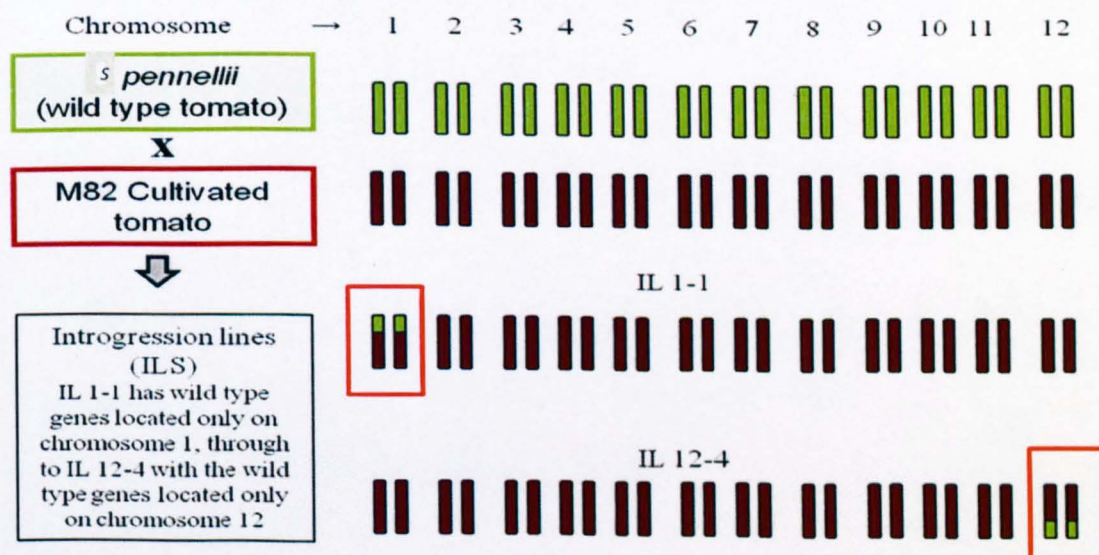


Figure 2-16 Schematic representation of the *Solanum pennellii* introgression lines, with background genetic material from *Solanum lycopersicum* CV M82.

[Introgression lines (ILs) 1-1 and 12-4 shown as examples, with the *S. pennellii* (wild type tomato) genetic material on chromosome one and twelve, respectively. . Adapted from (Zamir 2012).]

The ILs were initially produced in a laborious process by Eshed and Zamir (1995) by successive introgression backcrossing and marker-assisted selection to create recurrent parent lines with single introgressed segments, Figure 2-17. Genetic markers delineate the position of the wild type chromosome fragment within the chromosome. A complete IL population reconstitutes the donor parent genome in overlapping chromosomal segments, that is the ILs combined have complete coverage of the wild species genome. If a favourable trait, such as increased fruit firmness were to be expressed on fruits from the IL2-3 for example, this indicates that a QTL for this trait is harboured by the introgressed region, and then further crosses can be conducted to break up the IL further and pinpoint the gene responsible for that trait. Tomato ILs have been used to identify numerous QTL (Lippman et al, 2007) including those that enhance fruit texture (Chapman et al. 2012) and control ethylene production during ripening (Dal Cin et al. 2009). Figure 2-18 shows the genetic parents (*S. pennellii* and *S. lycopersicum*) and six of the ILs with easily distinguishable differences in colour and carotenoid content (Zamir 2001) as an example of some of the variation occurring in the ILs.

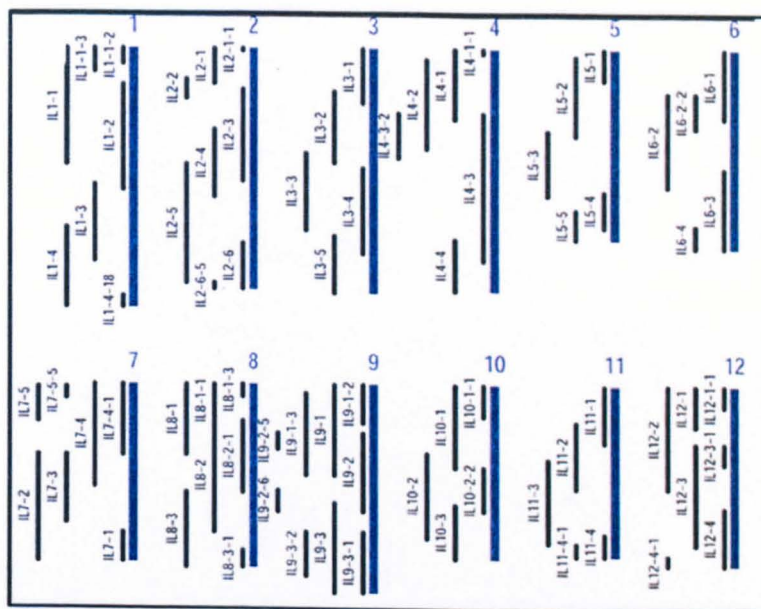


Figure 2-17 Genome introgressions on the 12 tomato chromosomes of the 76 *S. pennellii* ILs. [Note that the ILs are nearly isogenic to each other, only differing at the marked introgression chromosome segments. Figure from (Lippman, Semel and Zamir 2007).]

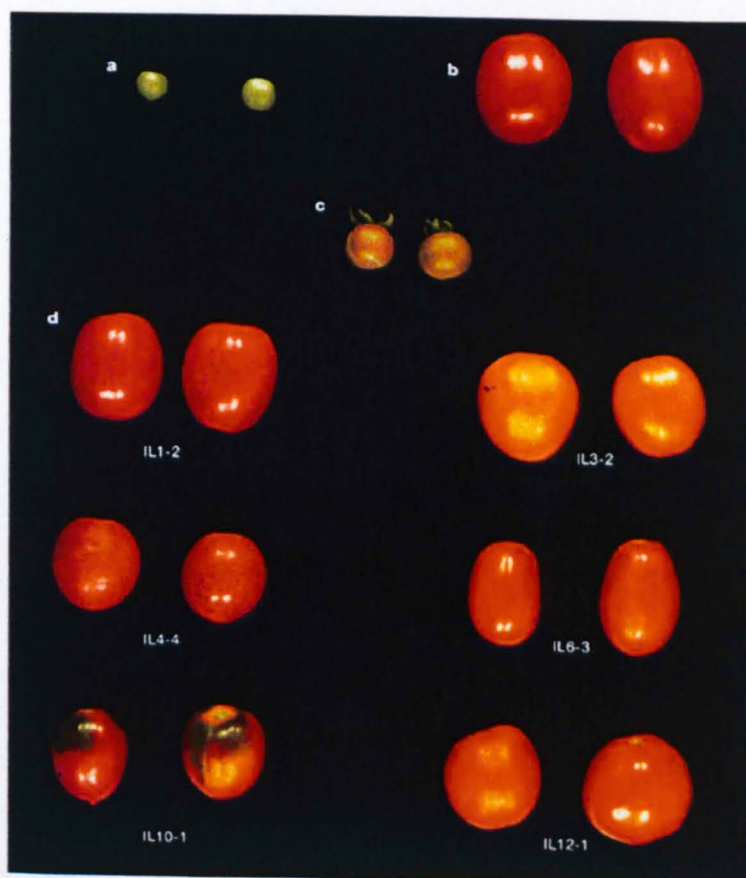


Figure 2-18 Example of the resultant differences in the introgression lines produced by crossing *S. pennellii* with the cultivated M82 (Zamir 2001). [(a) Green fruits of *S. pennellii* (wild species); (b) Red fruits of the lycopene-rich *S. lycopersicum*; (c) their F1 hybrid progeny; and (d) six introgression lines (ILs) with different fruit colours and carotenoid –content phenotypes. Each IL contains a single, wild-species derived chromosome segment containing a gene that is affecting fruit phenotype.]

The identification of such phenotypes and linking it to a specific gene or area of DNA is however, not a straightforward task. For example, quantitative traits such as fruit size or colour development in ripening fruit vary continuously throughout the population. Viscosity poses challenges because it can be described as a complex trait, and is quantitative or polygenic. It is influenced by multiple genes found in disperse areas of the genome, as indicated by the number of enzymes involved in the formation and subsequent breakdown of the cell wall during fruit development. The ILs provide a way to Mendelise a complex trait by allowing a focus on one component situated in a specific area of the genome.

2.5 MEASUREMENT OF RHEOLOGICAL PROPERTIES OF FRUIT MATERIALS

2.5.1 Texture of the fruit

There is an assumption that texture of the fruit is related to the quality of the pureed material. A compressive texture test can be used to assess the texture of fruits for comparison with the rheology of the fruit when pureed. Powell et al. (2003) related the effects of fruit texture on viscosity of puree of tomatoes with co-suppressed ripening related genes, Expansin (LeExp1) and PG gene (LePG). The co-suppression of these two genes resulted in firmer fruits and higher viscosity of the puree. Powell et al. (2003) also reported a loss of viscosity on ripening using a Bostwick consistometer to compare the viscosity of hot break puree of Ailsa Craig tomatoes over a range of maturity, mature green/ breaker, pink and red tomatoes.

This assumption may not be the case since a hierarchy of structures contribute to the texture of a fruit, and a combination of factors affects the rheology of pureed fruit. For example whole fruit firmness can be quantified by measuring the force required to physically compress the fruit. Compression occurs between two flat plates and the force-relaxation characteristics can be followed for the duration of the test and provides some information as to the viscoelastic response of the fruit. This kind of whole fruit measurement will be influenced by the mechanical properties of the cuticle, as has been demonstrated by (Matas et al. 2004), as well as the firmness of the pericarp tissue, and the proportion of the locular gel. These tissues in turn are composite structures of cells and cell walls (Prasanna, Prabha and Tharanathan 2007), and at the cellular and tissue level, the dominant factors involved in the softening of fruit during ripening are shown in Table 2-2.

Table 2-2 Dominant factors in the texture of fruit tissue	
Factor	Scale
Cell-cell adhesion	~1mm (i.e. a few cells together)
Cell wall rigidity	~1µm
Turgor	0.5mm (Single cell behaviour)

A needle-like probe can be used instead of two large flat plates to gain information about the texture of a tissue, thus omitting the top level (the whole fruit) of the hierarchy shown in Figure 2-1. A thin needle probe of 2 mm in diameter, as used for the textural tests for tomatoes of different ripeness in this current work, exerts the force over a smaller area of the tomato. It can measure the properties of an area of tissue formed from relatively few cells (assuming that a 2mm diameter probe will

theoretically come into contact with individual cells of approximately 200-500µm in diameter).

The effects of a compressive test on the turgor, cell to cell adhesion and cell wall rigidity of plant tissue at the microscopic level have been described by Bourne (1983). A cell is deformed in the direction of the applied load, and because a cell is relatively incompressible, the cell surface to volume increases. This causes a stress on the cell wall, which causes an increase in turgor pressure of the cell. The difference in turgor pressure between a compressed cell and the apoplast is re-balanced by a movement of water until the internal and external water potentials are equalised. Distension of the cell wall and deformation of the middle lamella occurs and so the cell to cell contact area is modified. At the end of the compression test, the external load is removed, and depending of the elasticity of the cells' wall, the cell is free to return to its original shape and recover its strain. The mechanical properties of the tissue show a time dependency due of the time taken for the movement of water returning to the cell, and to the time dependent properties of the cell wall.

The effect of turgor pressure is lost when fruits and vegetables are heated during cooking. This is due to the degradation of the cell membrane (Greve et al. 1994).

2.5.2 Rheology of the puree

During the processing of fruit into puree, the tissue can break up (i) between cells, at the middle lamella, (ii) across the cell wall itself or (iii) a combination of the two

to form particles. It follows that the likelihood these scenarios would be dependent on the integrity and mechanical strength of the middle lamella and the cell wall. Cells that are damaged or ruptured release their contents to form the serum in which the particles are suspended in and hydrated by it. The water soluble pectic material is released from the cell wall and middle lamella into the serum, and some remains in the insoluble particles.

Assuming that tomato puree is a suspension/dispersion, and not sub-micron sized (and therefore colloidal factors are assumed not significant), the factors affecting the contribution of the particles and of the serum to the rheology of the puree could be listed as Table 2-3:

Table 2-3 Factors affecting the rheology of puree (Barnes 2000)

Particles	Serum
Concentration	Viscosity
Shape	Pectin/polymer content
Size and size distribution	
Particle-particle interaction	
Particle deformability	
Charge	
Particle – serum interaction	

One of the key quality parameters of tomato puree is its viscosity. In addition measurement of the rheology of the materials gives insights into its composition

and physicochemical behaviour. From the information given in this chapter it has been identified that the puree can be considered to consist of three elements:

1. Low molecular weight materials, such as fructose
2. High molecular weight water soluble hydrocolloids e.g. pectins
3. Water insoluble cell fragments, whole cells and clusters of cells

Critical for the perceived viscosity will be the concentrations of these materials. The viscosity will be dependent of the hydrodynamic volume of each of the three categories of materials listed above. The radius of a small dissolved sugar is in the region of 1nm, while for a hydrocolloid this may be 50nm (Diaz et al. 2009). The average radius of the particles in a tomato puree was in the region of 200µm and therefore the sizes of the materials creating the overall purees differ widely. This is relevant because the concentration and the volume occupancy of the materials affect the viscosity.

In a dilute solution where there is a lot of solvent and low amounts of the three elements (small soluble sugars, soluble hydrocolloids, or insoluble particles) they can move independently from each other. In the case of low concentration of the soluble polysaccharide coils in isolation, viscosity is generated by the random coils tumbling in the flow of the solvent and therefore viscosity is dependent partly on molecular weight. As concentration increases, a point is reached where individual coils are so close to adjacent coils that entanglement occurs and viscosity increases greatly. This is known as the coil overlap concentration, c^* as shown in Figure 2-19.

As concentration is increased above the c^* the viscosity shows a further increase, as the coils are forced to entangle further.

Hydrocolloid shape and interactions will be affected by the charge carried by the macromolecules, the assemblies of the molecules and the ionic nature of the solvents.

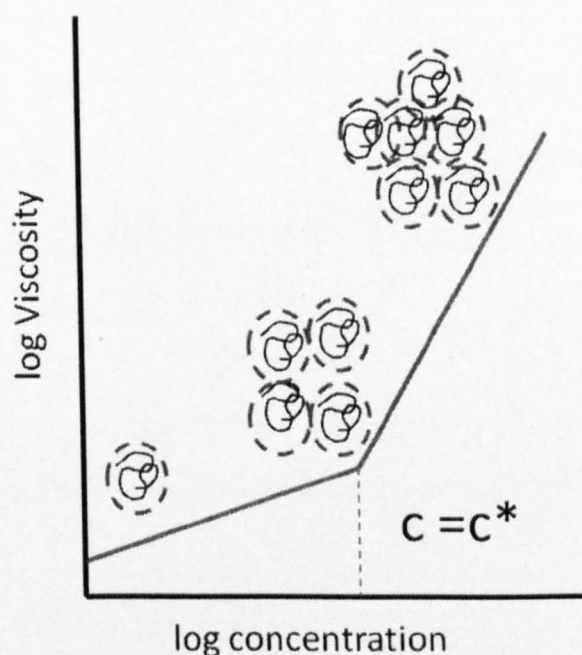


Figure 2-19 Concentration dependence of soluble hydrocolloids – the concept of c^* concentration and the effect on viscosity

If the particles are non-deformable and they are in suspension their behaviour can be described in dilute, intermediate or concentrated environments. Each of these is now considered individually, starting with dilute suspensions of solid spheres.

Einstein in 1906 and 1911 stated that for solid spheres ($\phi \leq 0.01$), assuming no interactions, the equation below can be used to describe viscosity of the suspension;

$$\eta = \eta_o(1 + 2.5 \phi)$$

Equation 2-1

Viscosity η is the measured viscosity of the suspension, η_o is the viscosity of the continuous phase, ϕ is the volume fraction occupied by the particles and 2.5 is assumed to be the $[\eta]$ the intrinsic viscosity, for spheres. At this stage the particles are considered weightless and are widely spaced; so much so that no interactions occur. Because of this particle size has no effect on the viscosity of the suspension.

For suspensions of intermediate concentrations (spheres ϕ between 0.01 and 0.05), hydrodynamic interactions between the particles (where particles are close enough that the flow around one is influenced by the presence of another) also affect the viscosity of the suspension, the equation below is true;

$$\eta = \eta_o(1 + 2.5\phi + K\phi^3) \quad \text{Equation 2-2}$$

Where k is a constant value.

The rheological behaviour of plant suspensions such as tomato puree is often based on the semi-empirical Krieger-Dougherty equation (Krieger and Dougherty 1959) which describes the behaviour of more concentrated suspensions, is shown below;

$$\eta = \eta_o \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m} \quad \text{Equation 2-3}$$

$$\text{or} \quad \eta_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m} \quad \text{Equation 2-4}$$

Where ϕ_m is the maximum packing fraction and η_r is the relative viscosity of a suspension. The relative viscosity η_r of tomato puree is related to the viscosity of the serum (continuous medium) and the viscosity of the suspension, as described below;

$$\eta_r = \frac{\eta_{\text{viscosity of suspension}}}{\eta_o \text{viscosity of continuous medium (serum)}} \quad \text{Equation 2-5}$$

The concept of the maximum packing fraction is of particular interest for the current work. For solid spheres, the maximum packing fraction is assumed to be ϕ_m 0.62. Deviation from the spherical shape results in a greater intrinsic viscosity $[\eta]$ due to the increased disruption of the flow field, and consequently a lower maximum packing fraction ϕ_m due to poorer space filling. Intrinsic viscosity is lowest for spheres, and higher for oblate (disc like) particles such as red blood cells, and higher still for prolate (rod-like) particles such as fibres. However, the major deviation from these theories comes from the feature that the insoluble particles in tomato puree are not *solid* spheres and are deformable. The deformable nature of tomato puree particles and the effect on viscosity will be discussed further in Chapter 6.

The particle size distribution also affects the maximum packing fraction.

Polydispersibility increases the ϕ_m because the smaller particles can fill the voids between the larger ones. These main factors that affect the maximum packing fraction are summarised in Table 2-4.

Table 2-4 Summary of parameters affecting the maximum packing fraction of particles.

'Poor' inefficient packing	'Good' efficient packing
non-spherical	spherical (spheres have the lowest intrinsic viscosity)
solid particles	deformable particles (fewer/smaller voids between deformable particles)
monodisperse particles	polydisperse sized particles (smaller particles fill the gaps between the larger ones)
lower shear rate	higher shear rate (‘improved’ packing configuration at higher shear rates)

The properties of the particles in tomato puree have been shown to affect viscosity in a range of studies. For example Ouden and Vliet (1997) concluded that the tomato concentrate particles were flexible (deformable); after wet sieving tomato concentrate, the particles were considerably larger than the pore size of the sieve. Studies on suspensions of tomato pulp of narrow size distributions (Yoo and Rao 1994) also obtained by wet sieving, showed that apparent viscosity decreased with average particle size. These two examples show how viscosity can be affected by the insoluble particle characteristics. In other studies, it has been shown that particle phase volume and the shape and size distributions of particles are also important see schematic shown in Figure 2-20.

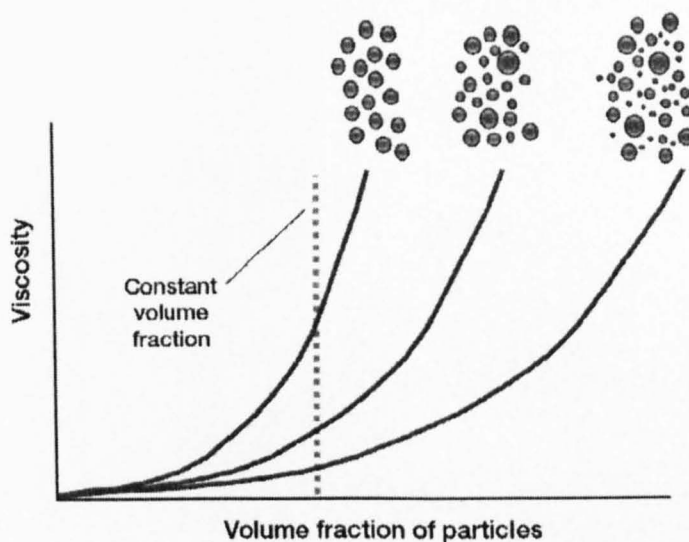


Figure 2-20 Effect of particle size distribution on volume fraction and viscosity of the suspension

2.5.2.1 Viscosity and shear behaviour

Another factor that affects the perceived viscosity is the response to shear. Tomato puree samples are subject to different shear regimes during manufacture, which may alter the particle state, and also samples are measured for viscosity when being sheared. Figure 2-21 shows the classic responses materials have when sheared. Small molecules, such as the simple sugars in the serum, are unlikely to show shear or time dependence and therefore can be classed as Newtonian.

Hydrocolloids, such as pectin are expected to show shear thinning. The behaviour of the particles could vary considerably when sheared. Each particle could change in shape as well as altering the packing behaviour. What will be important are their shape, flexibility and interconnectivity (particle –particle interaction or particle-polymer interactions). The values for viscosity and understanding of the rheology of the pastes will be dependent on the methods used to study the tomato puree.

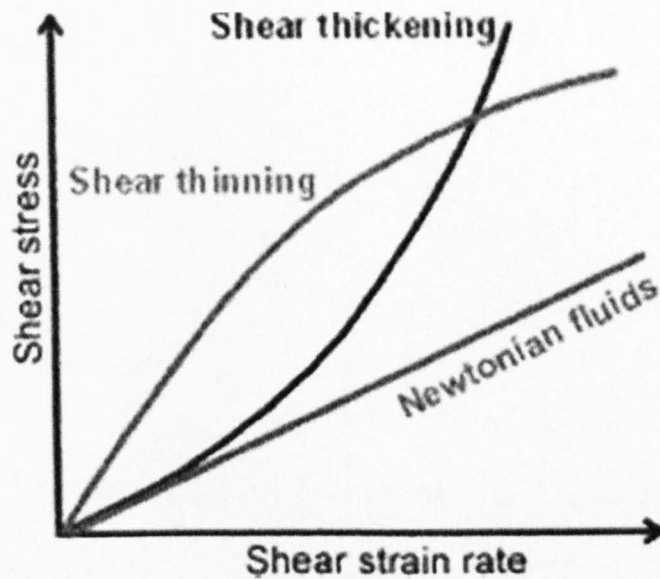


Figure 2-21 Behaviour of Newtonian, shear thinning and shear thickening fluids

2.5.2.2 *Measurements of viscous and elastic behaviour*

Rotational viscosity

Viscosity is a physical characteristic of all liquid materials. It can be defined as the internal friction of a fluid and is a measure of resistance to flow. It is possible to measure the viscosity of samples between two surfaces, or plates whereby the upper of these surfaces moves in one direction whilst the lower one is stationary.

The sample, located between the two plates is therefore subject to shear. The basic terms relevant to shear rheology are explained with Figure 2-22.

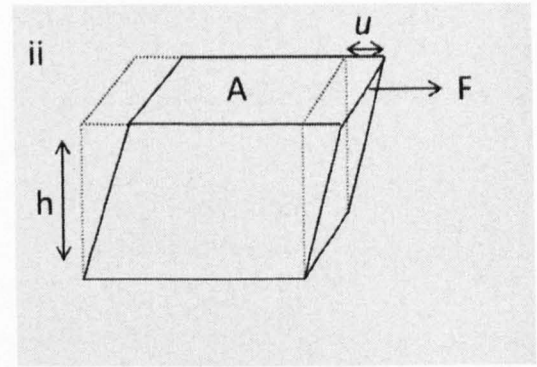
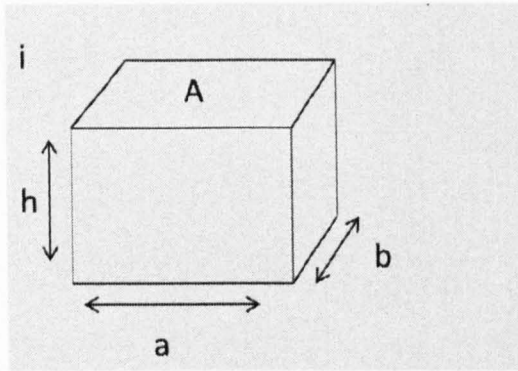


Figure 2-22 Definition of shear terms (see text below for further explanation)

Figure 2-22 (i) depicts a cube of material with dimensions $a * b * h$. The upper surface (A) has the area of $a * b$. In figure (ii) it shows that when a force (F) is applied across upper surface A, then the material will deform slightly. Shear stress, shear strain and shear rate can be defined in the following way;

$$\text{shear stress } (\sigma) \text{ Pa or N/m}^2 = \frac{F \text{ (force in N)}}{(\text{surface area in m}^2)} \quad \text{Equation 2-6}$$

$$\text{shear strain } (\gamma) = \frac{u \text{ (deformation)}}{h \text{ (height)}} \quad \text{Equation 2-7}$$

$$\text{shear rate } (\dot{\gamma}) \text{ (s}^{-1}\text{)} = \frac{\text{change in strain}}{\text{change in time (s)}} \quad \text{Equation 2-8}$$

This can be visualised as the movement of 'layers' sliding over each other (laminar flow). In a rotational viscometer, the shear rate is derived from the rotational speed of the upper plate (e.g. the upper parallel plate). The amount of shear is dependent on the gap and the rate of movement of the mobile plate. The rate of shear is imposed on the sample and the resultant strain can be measured. Shear viscosity is then defined as shown below;

$$\text{shear viscosity } (\eta) = \frac{\text{shear stress } (\sigma)}{\text{shear rate } (\dot{\gamma})} \quad \text{Equation 2-9}$$

A typical viscosity dependence on shear rate curve for a tomato puree is shown in Figure 2-23 and the relationship between shear rates and stress are shown for the same data set in Figure 2-24.

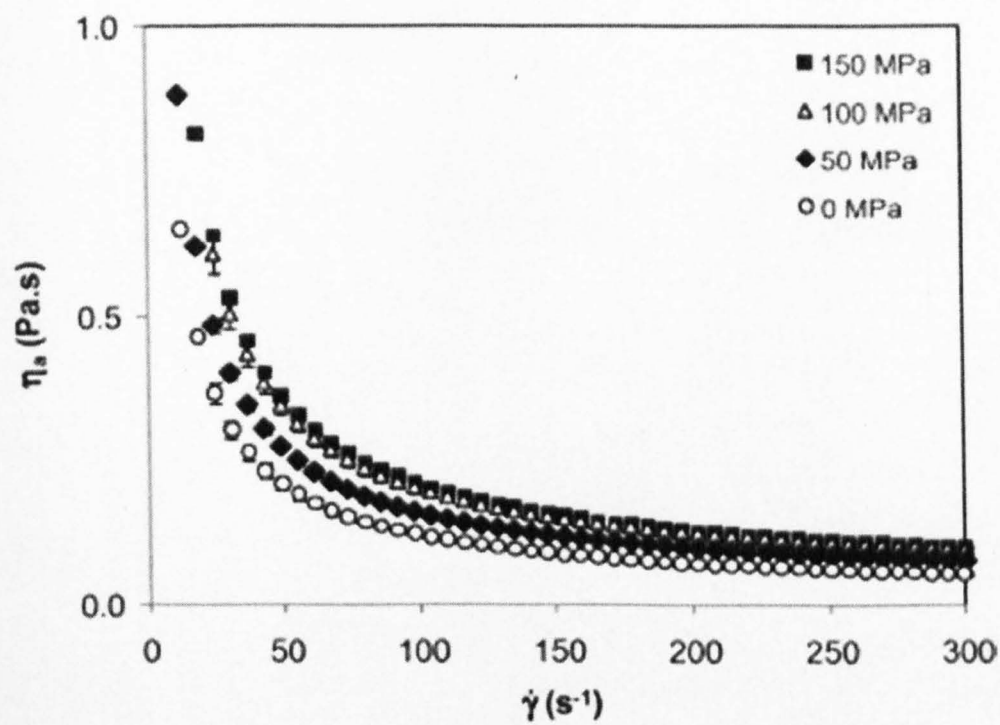


Figure 2-23 Flow curves showing viscosity, measured at 25C, at different shear rates for tomato puree processed with high pressure homogenisation (Augusto, Ibarz and Cristianini, 2012).

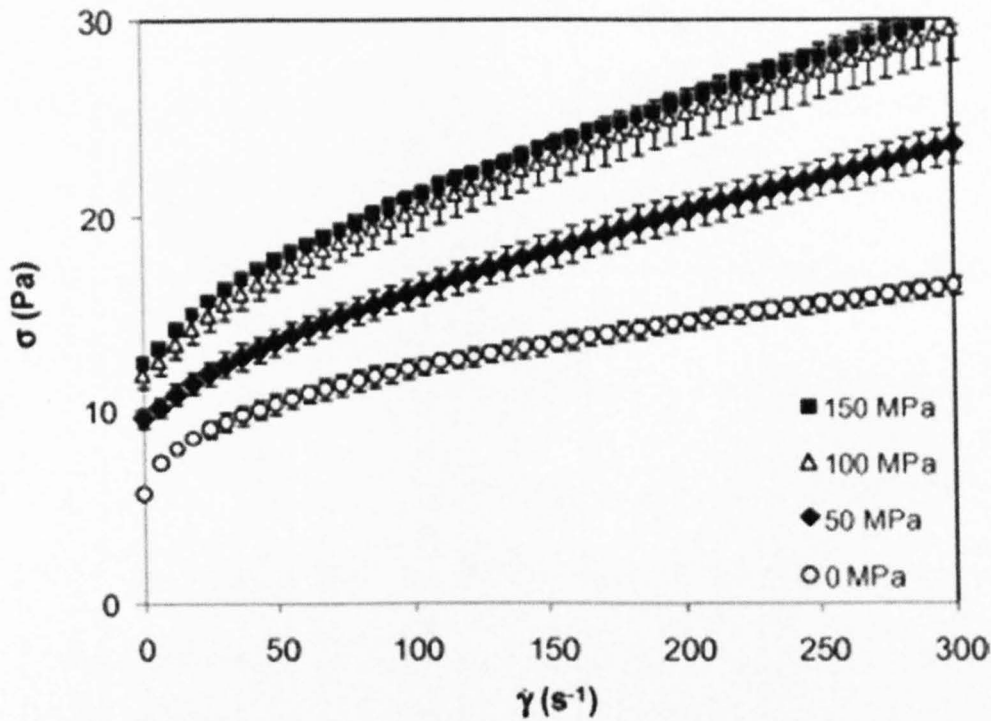


Figure 2-24 Relationship between shear rate and stress for tomato puree processed by high pressure homogenisation (0-150 MPa). Mean of three replicates at 25°C, vertical bars represent the standard deviation for each value (Augusto, Ibarz and Cristianini, 2012).

Typically, applying a wider shear regime (low to high shear rate) the flow behaviour of tomato suspensions show apparent viscosity values with an initial Newtonian plateau region followed by a shear thinning region. A power law model (below) can be used to describe the shear thinning flow behaviour of tomato puree;

$$\sigma = K \dot{\gamma}^n \quad \text{Equation 2-10}$$

σ is shear stress, K is the consistency index and $\dot{\gamma}$ is the shear rate and the exponent n (dimensionless) is the flow behaviour index. The flow behaviour index n is a value that can be used to describe the flow behaviour compared to Newtonian flow. An n value of 1 is a Newtonian fluid, <1 is shear thinning, >1 is shear thickening.

Tomato purees are shear thinning and have been shown to have n values of 0.113 to 0.208 (Xu, Shoemaker and Luh, 1986). As reviewed in (Rao, 1999) the consistency index (i.e. the K value) is affected by the solids concentration in the puree. The empirical nature of the power law model should be noted, it does not describe the behaviour at very low or very high shear rates.

A factor that would be expected to be important in the rheological behaviour of the purees is if the samples had undergone shearing prior to measurement. Many samples show a time dependent rheological behaviour. Figure 2-25 shows the fall in shear stress as a sample is sheared (at 300 s^{-1}) for increasing lengths of time, up to 600 seconds. The more viscous samples show a marked decline in shear stress at the start of the shearing process. However, after 100 seconds no further decline in the viscosity was apparent. This behaviour was looked at for the tomato purees used in the experimental work reported in this thesis, and to reduce the random pre shear behaviours, all the samples were pre-sheared for 90 s at 10 s^{-1} between each measurement (see section 5.2 for further details).

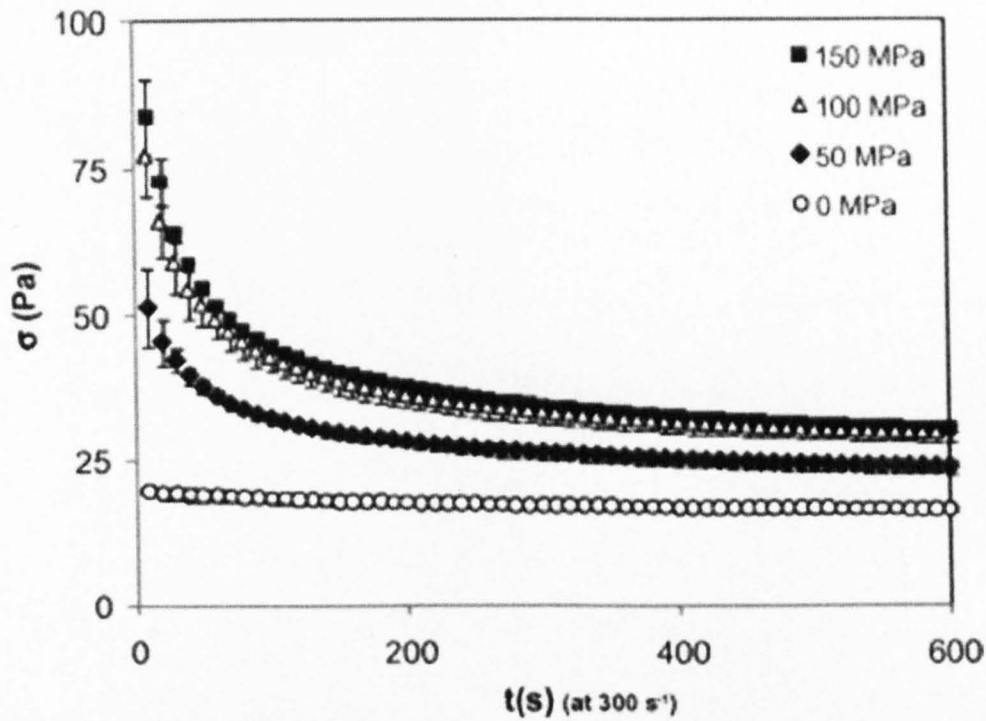


Figure 2-25 Tomato puree samples processed by high pressure homogenisation (0-150 MPa) (as shown in Fig. 2-23 and 24) showing initial sensitivity to shear regime (Augusto, Ibarz and Cristianini, 2012).

Gap size and homogeneity of the sample are critical for fundamental rheology measurements. As the tomato pastes consist of a range of materials, which could segregate during the measurements, and as the materials may 'slip' during shearing it is possible to use measurement geometries that would give more reproducible data, although some of the fundamental interpretation of the rheology would be lost. In the experimental work for this thesis measurement geometries of double gap, cone and plate and serrated plate have been used, but most of the reported data will be for the latter serrated plate. These geometries are described in Chapter 3.

Dynamic rheology

The shear regime can cause damage to the microstructure of the sample measured, this affecting shear viscosity. In addition, flow characterisation tests are used to measure a materials' response to an imposed constant shear rate or shear stress. They give information only about the materials *viscous* properties (resistance to flow). To gain information about a material's viscous and elastic properties, dynamic or oscillatory measurements should be made. An example showing the measured moduli, storage G' and loss G'' , for tomato puree at different strains is shown in Figure 2-26. In depth mathematical explanations of these moduli, values can be found in Barnes (2000). The complex modulus, G^* , G' storage (elastic) modulus and the G'' loss (viscous) modulus can be defined as shown below.

$$\text{complex modulus } G^* = \frac{\text{stress}}{\text{strain}} \quad \text{Equation 2-11}$$

$$\text{storage (elastic) modulus } G' = \frac{\text{stress}}{\text{strain}} \times \cos(\text{phase angle}) \quad \text{Equation 2-12}$$

$$\text{loss (viscous) modulus } G'' = \frac{\text{stress}}{\text{strain}} \times \sin(\text{phase angle}) \quad \text{Equation 2-13}$$

Definitions of materials as being fluids or gels are often made on the ratio of G'/G'' . Tomato purees and pastes have been described as having a weak gel like behaviour, in that the magnitudes of G' were higher than G'' with both increasing with oscillatory frequencies (Rao and Cooley, 1992).

Linear viscoelastic region

Figure 2-27 indicates that as the rotational distance of the upper plate of the measuring system, in reference to the other increases (strain sweep), there is change in both the elastic and viscous moduli, known as G' and G'' . However, G' is independent of the strain at the lower values and this is known as the linear viscoelastic region (LVR). The length of the linear elastic region is often associated with the particle nature of materials. For example in emulsions the length of the region can be associated with the size of the disperse phase. The other feature often seen is if the sample is particle in form then there can be an increase in G'' at the end of the LVR.

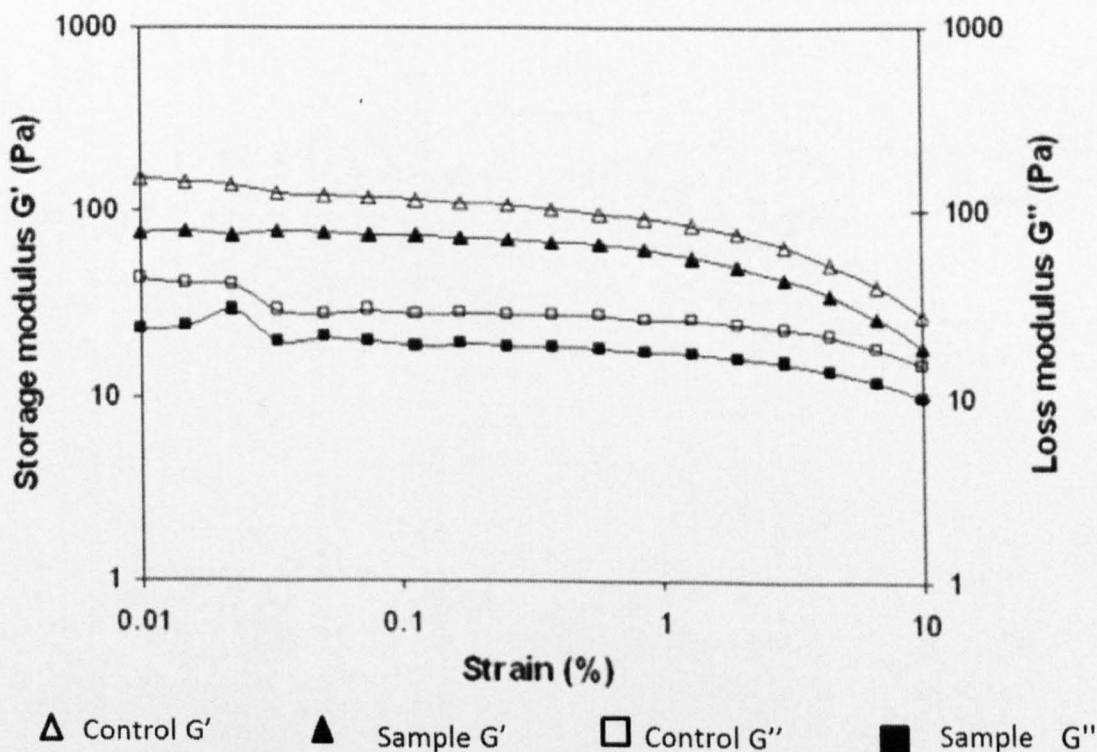


Figure 2-26 An example of an Amplitude (strain) sweep to determine the extent of the linear viscoelastic region (LVR) of 2 homogenised tomato purees at a constant angular frequency of 10 rad/s at 20°C.

[The sample had been heated to 50°C for 15 minutes before the strain sweep measurement. The LVR is the region where G' is independent of the strain. Taken from Verlent et al (2006)]

To establish the linear viscoelastic region a strain sweep (as in example shown in Figure 2-26) is carried out and a strain selected that falls well within the linear region. Using this strain a frequency sweep can then be carried out. An example is shown in Figure 2-27.

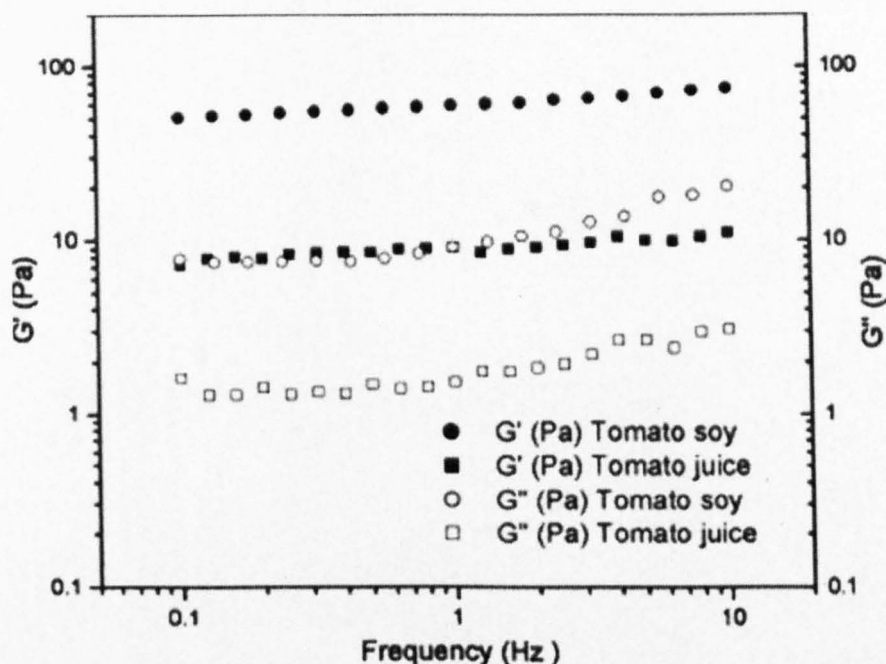


Figure 2-27 Frequency sweep of tomato puree with and without soy protein, with G' and G'' as a function of frequency at 1% strain (taken from Tiziani and Vodovotz, 2005)

Linking rotation and dynamic rheology.

As the rotational and oscillation of the samples both relate to a rate of deformation it should be possible to equate these two forms of fundamental measures, if the shear forces are equivalent. This is normally evaluated using the empirical Cox Merz rule (Cox and Merz 1958) which states that the complex viscosity η^* (see below) and the steady shear viscosity η are equivalent when the angular frequency ω is equal to the steady-shear rate $\dot{\gamma}$, below.

$$\eta^*(\omega) = \eta(\dot{\gamma}) \text{ when } \omega = \dot{\gamma}$$

Equation 2-14

Where the complex viscosity η^* is;

$$\text{complex viscosity, } \eta^* = \frac{G^*}{\text{frequency}}$$

Equation 2-15

It is reported that the Cox Merz rule holds for many polymeric samples, but there are many samples where the relationship between the dynamic and steady flow rheology cannot be superimposed (Alhadithi, Barnes and Walters 1992). For the tomato samples measured and reported in this work it should be remembered that serrated plates were typically used and this would impact on the practical shear regimes experienced by the product.

Empirical measures of tomato rheology

Fundamental assessment of viscosity requires knowledge of the shear regimes applied to the samples and the sample needs to be homogenous. Often this is not possible for systems such as purees as the different compositional material can segregate during the measures. For the rotational and dynamic viscosity serrated plates needed to be used and this would have invalidated a true fundamental interpretation of the rheological data. It is therefore reasonable to consider measurement of such complex systems using an empirical technique.

A viscosity that measures the torque required to keep a paddle stirring allows a precise and easy way to record an empirical value for viscosity. An adapted method

using a Rapid Visco Analyser (RVA) for measuring tomato purees provide a stirring regime that does not allow the particles to sediment, while the shear is insufficient to cause major particle size reduction. The paddle will exert a range of shear forces, with those at the tip of the paddle blade being much greater than the forces at the centre. The average shear forces for an RVA paddle have been calculated (Lai, Steffe and Ng 2000). The ranges across the blade however were found to be several decades.

Despite the problems of difference in shear, an empirical technique has sufficient benefits of reproducibility and sample discrimination to be a useful technique for looking at the tomato purees. The RVA was used extensively for the results shown in this thesis. Measurement of tomato purees' viscosity is therefore a compromise between gathering information about the behaviour in an understandable format, and using the data for direct comparisons between samples.

2.6 AIMS AND HYPOTHESES

The effect of tomato ripeness on the texture of the fruit and material properties of the particles and the soluble material once pureed, and how these consequently affect the viscosity of the puree is not fully understood. Therefore the objective of the research described in this thesis was to provide an understanding of the parameters that determined the quality of tomato purees. To do this a series of specific aims were undertaken and these included:

- i) To source tomatoes of different ripeness and known genetic origin (*S. pennellii* Introgression line) and to establish if ripeness or known genetic changes relate to the properties of the puree.
- ii) To establish reproducible methods for the creation and measurement of tomato purees that can be used to screen materials and for more in depth study.
- iii) To understand the features of pureed material in terms of its rheological behaviour.
- iv) To establish if there is a relationship between texture of tomatoes and the pureed material.

These aims were to test a series of hypotheses that included;

- i) Factors associated with ripeness, including texture of tomato pericarp correlate with the viscosity of the pureed pericarp tissue. This was investigated in chapters 4, 5, 6 and 7)

- ii) The viscosity of tomato puree is dominated by the suspended solid fraction (the particles) rather than the total solids or the serum viscosity.(chapters 6 and 7)
- iii) Tomato fruits can be collected, prepared into a puree and then the viscosity of the puree measured. A simple stirred viscosity test can be conducted on the tomato purees so that correlations can be made between texture of the fruit and viscosity of the puree (chapters 4, 5, 6 and 7).
- iv) At the same stage of ripeness, tomato purees prepared from tomatoes from the *S. pennellii* introgression library differ in viscosity (chapter 7).

CHAPTER 3: MATERIALS AND METHODS

3 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Commercial tomato juice (puree)

Sainsbury's basics tomato puree, made from concentrate was purchased from the local store in 1 litre cartons. The ingredients listed on the packaging were; tomato puree from concentrate (98%), lemon puree from concentrate and salt. It was stored unopened at room temperature and at 4°C after opening. Two batches of Sainsbury's puree with different production codes were analysed and compared, they are referred to as X1 and X2 in this report. Sainsbury's tomato puree had a total solids level comparable with the purees prepared from the fresh commercial tomatoes and IL tomatoes.

3.1.2 Commercial tomatoes

Commercial tomatoes were donated by Evesham Vale Growers (Evesham, UK). They were separated by colour into four groups namely; mature green, breaker (turning from green to slightly pink), pink and red ripe on the day of delivery. Ten of each were randomly selected and colour and texture measurements were taken. Measurements were taken for skin colour and pericarp texture before the locular tissue was removed. The tomato pericarp halves with skin on were kept under frozen storage conditions (-80°C) until processed into puree.

For preliminary work done on the viscosity screen for the *S. pennellii* introgression lines, commercial red ripe tomatoes were purchased from the local supermarket, fruits were sliced in half and locular tissue removed before storing the pericarp halves with skin on under frozen storage conditions (-80°C) until processed into puree.

3.1.3 *S. pennellii* introgression lines

Tomatoes from the *S. pennellii* introgression lines were harvested at breaker plus 7 days from the glass house at the University of Nottingham. Three replicates of 54 lines out of the complete range of 76 were harvested, and the control parent line M82.

3.1.4 Reagents

Reagents used in the experimental work were analytical grade and purchased from Fisher Scientific, unless otherwise stated, such as sucrose (granulated sugar, Tate and Lyle, purchased from the local supermarket) and sodium chloride, Acros Organics, analytical grade.

Sodium azide was added to all purees at a concentration of 0.04% to inhibit microbial growth, and puree was stored at 4°C for up to 3 weeks.

3.2 METHODS

3.2.1 Collection of the tomatoes from the *S. pennellii* introgression lines – viscosity screen

Three plants per line (76 lines in total) and six plants for the controls: wild type *S. pennellii* and M82, were grown from seed in a heated glass house under controlled conditions at Sutton Bonington. Glass house conditions were as follows; 16 hours of daylight, day temperature 20°C, night temperature of 18°C. Plants were grown in 7.5l pots of Levington M2 pot/bedding compost, and daily irrigation was supplemented with fertiliser. The location of each plant was randomised over 17 rows of up to 15 plants per row. The tomatoes were harvested between June and July 2010 by hand as individual fruits ripened. Fruit from the first truss were not harvested. Fruit were tagged at breaker stage, and harvested 7 days later. Breaker stage was defined as the fruit being half green half pink, the first visual indication of onset of ripening.

3.2.2 Collection of the tomatoes from the *S. pennellii* introgression lines – firm fruited line 3-4 and control M82

Fruits were harvested from tomato plants grown in the glass house, as described in 3.2.1 from one plant of M82, and one plant of IL 3-4, a line found to have firm fleshed fruit. These plants were grown and harvested in the winter of 2009-2010. They were harvested at four stages of maturity, the mature green stage (40 DPA (days post anthesis), at the breaker stage, breaker plus 7 days and breaker plus 10 days. Results of the experimental work carried out on these fruits are described in

section 7.2.7. Four fruits per stage were harvested and results of fruit skin colour and texture were recorded. The colour of fruit skin and pericarp texture measurements were carried out as per sections 3.2.3 and 3.2.4.

3.2.3 Colour Analysis

Tomato colour was measured using the Minolta Chroma meter CR-400 (Konica Minolta, Osaka, Japan) using the CIE $L^*a^*b^*$, (lightness/green-to red scale/blue-to-yellow scale) colour space with the a^* chromaticity coordinate indicating a colour change with values negative to positive expressing the colour change from green to red. For the commercial tomatoes, and the study to compare a firm fruited IL 3-4 with the control M82, the colour of the skin was recorded. Three different locations on the fruit were measured on the skin per fruit. For the viscosity screen colour measurements were taken of the fruit puree.

3.2.4 Texture measurements of pericarp tissue from the commercial tomatoes and the study to compare firm fruited IL3-4 with the control M82

To assess firmness of the fruit tissue, maximum load values were recorded using the Ametek Lloyd LF PLUS texture analyser (Fareham, UK) equipped with a 10 N load cell on a 6 mm transverse section cut from each fruit (ten fruits at each stage of ripeness, two replicates on the inner and outer pericarp tissues were taken per fruit). A 1.6 mm diameter flat head cylindrical probe was moved 4 mm into the pericarp at 10 mm/min and the maximum load (the force required to penetrate the

pericarp tissue at 10 mm min^{-1}) was recorded. Typical results and procedure are shown in Figure 3-1; the probe as it is about to enter the tomato. The sample being assessed is a mature green fruit and the green pigmentation can be seen throughout the flesh. As the small probe penetrates the flesh the force required to push the probe through the cells is measured. An example of the force penetration curves for two different samples is given in Figure 3-2 , breaker and red ripe. The maximum load represents the greatest load (N) required to cause failure of the tissue integrity, with a higher maximum load indicating a firmer fruit.

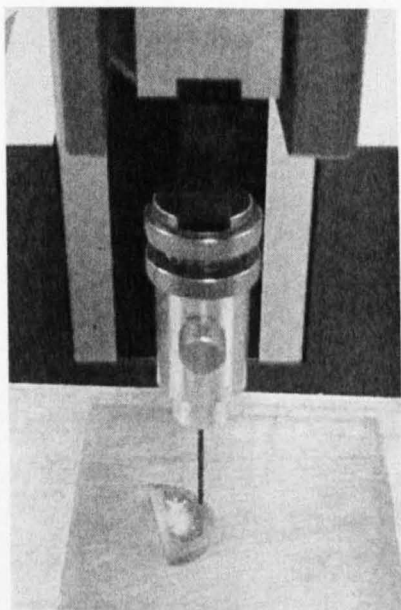


Figure 3-1 Firmness measurement on a transverse section of a mature green tomato fruit using the texture analyser

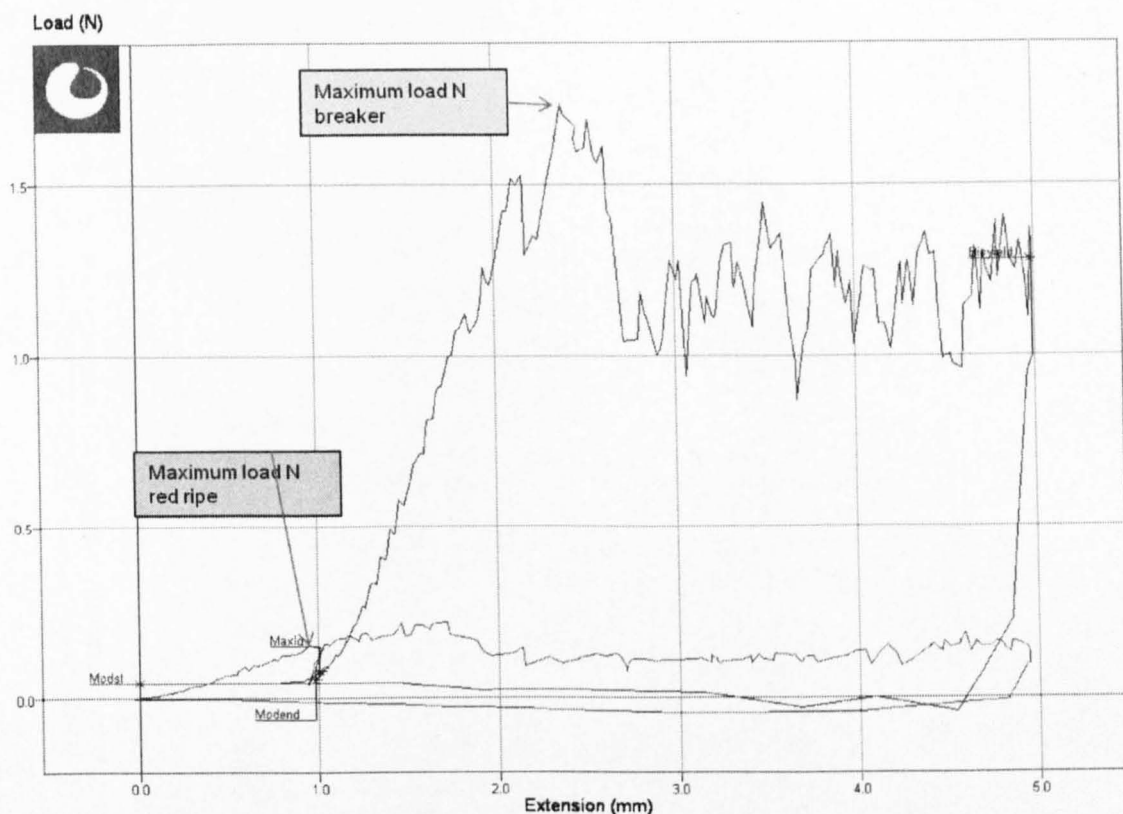


Figure 3-2 Typical texture data for breaker and red ripe outer pericarp, with maximum load (N) shown

3.2.5 Storage of tomatoes before pureeing

Within 2 hours of harvesting, the IL tomatoes were frozen, first being washed in distilled water and dried with tissue paper. The fruits were sliced in half through the stem and seeds removed, and then the tomato was blotted with tissue paper and immediately frozen in liquid nitrogen. Tomatoes were stored at -80°C for no more than 4 months.

3.2.6 Preparation of puree

A simplified process based on commercial production practices (described in the literature review section 2.1) was developed to prepare puree from the pericarp tissue from tomatoes as part of the experimental work. A hot break process was

selected to inhibit the further break down of the pectins in the puree, and the sieving/screening process was omitted, because the breakdown of the particles on processing was of importance to the project. Preparation of puree was either in larger quantities, of approximately 150g for commercial tomatoes; for the IL tomatoes where individual fruits were pureed, 40g was made.

3.2.6.1 Larger quantities (150g) –commercial tomatoes

To make the puree, the tomatoes were peeled frozen and the pericarp tissue was cut into approx. 1cm³ cubes. In the more commonly used hot break process, samples were microwaved at 800W for four minutes, stirring half way through. The temperature was checked to be 95 °C +/- 2 °C for at least 5 seconds and enzymic activity assumed inhibited by this treatment. Samples were cooled to room temperature, and pureed with a 300W hand held blender (Sainsbury's) for three minutes on power setting 2. For the cold break method, cubes of the peeled frozen pericarp tissue were allowed to defrost at 4°C for 12 hours. Both hot and cold break tomatoes were then blended for the same length of time using the hand held blender

3.2.6.2 Smaller quantities (40g)-IL tomatoes

To make the puree, the tomatoes were peeled frozen and the pericarp tissue was cut into approx. 1cm³ cubes. Samples were microwaved at 800W for 1 minute and 20 seconds, stirring half way through. The temperature was checked to be 95 °C +/-

2 °C for at least 5 seconds and enzymic activity assumed inhibited by this treatment. Samples were cooled to room temperature and pureed with an Ultraturrax (IKA, T10) for 1 minute at speed 4.

3.2.7 Preparation of CDTA soluble pectins

Alcohol insoluble solids were prepared from commercial mature green and red ripe tomatoes. The pericarps were peeled and diced whilst frozen. Frozen tomato pericarp (100g) was immersed in liquid nitrogen and milled in a coffee grinder for 30 seconds. It was added to 500ml -20°C acetone, stirred briefly, and filtered and rinsed through Miracloth (Calbiochem) with 200ml 80%v/v acetone. It was added to 500ml of boiling 70%v/v ethanol, heated at 95°C for 10 minutes and cooled in an ice bath. The sample was filtered through Miracloth and rinsed with 500ml acetone. The residue collected was air dried overnight at room temperature. The resulting cell wall material was stored over P₂O₅ under vacuum at room temperature until required. Samples were prepared in duplicate.

To obtain the CDTA soluble pectins from the alcohol insoluble solids (AIS), a method adapted from Redgwell and Selvendran (1986), was used. The AIS was added to water (20mg/ml), stirred at room temperature for 6 hours and filtered through Miracloth. This filtrate contained the cell wall material that was water soluble. The water insoluble solids were then stirred at room temperature for 6 hours in a solution of 50mM CDTA (cyclo-hexane-trans-1,2-diaminetetra-acetate) and 50mM

sodium acetate (pH 6.5). The CDTA soluble material was collected by filtering through Miracloth, and then to remove the extraction reagents, dialysis of the filtrate was done against 5 l of water in 12-14 kDa dialysis membrane against four changes of water per day. Once dialysed, samples were freeze dried. Powdered cell wall extracts were then stored over P_2O_5 for 10 days to remove residual water. A 4% w/w solution of the CDTA soluble pectins was prepared by adding it to water, heating to 60°C for 15 minutes whilst stirring and then cooling to room temperature. 0.04% sodium azide was added to inhibit microbial growth.

3.2.8 Rheological characterisation of tomato materials

3.2.8.1 Rotational viscometry

A rotational rheometer (MCR301, Anton Paar, Graz, A) was used to obtain viscosity values of tomato puree and pelleted material at 25°C at 10 s^{-1} and 100 s^{-1} using a serrated parallel plate geometry (diameter 25 mm, gap height 1mm), Figure 3-3. Fruit purees and pulps are generally characterised as non-Newtonian fluids, as a result of their complex interactions between the particle and serum components, therefore a flow curve was acquired for the samples between $1\text{--}200\text{ s}^{-1}$ with an interval of 160s followed by a decreasing shear rate.

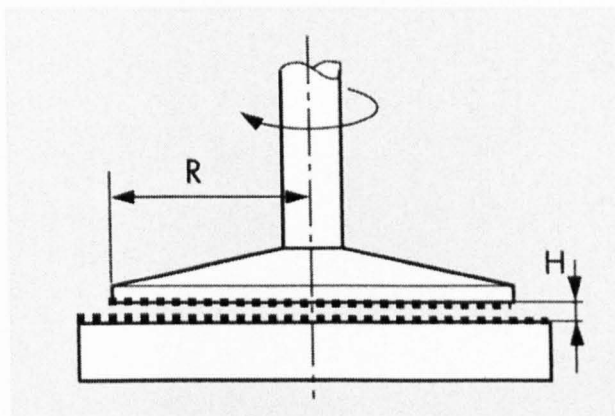


Figure 3-3 Diagram of the serrated parallel plate measuring system. H – height of gap was 1mm and R- radius was 125 mm. Diagram adapted from (Mezger, 2006) .

The double-gap measuring system Figure 3-4 was used to measure serum viscosity and sucrose solutions 0-20%w/w, since both types of sample were low viscosity liquids. A flow curve was obtained on the samples from $150\text{--}200\text{ s}^{-1}$ at 25°C , and separately at 10 s^{-1} for 90 seconds. Measurements were carried out at least in duplicate. The construct of the double gap geometry is designed to allow contact of the test material with the inner and outer surface of the bob. Due to this increased shear area, sensitivity is increased (compared with a concentric cylinder measuring system) and lower torques can be determined (Mezger, 2006).

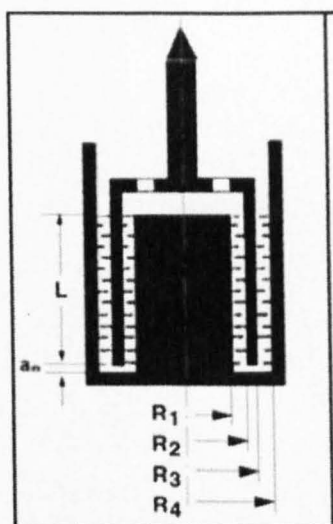


Figure 3-4 Schematic illustration of the double gap measuring system. R1-11.910mm, R2 – 12.328mm, R3 – 13.331mm, R4-13.8mm, L – 40mm.

3.2.8.2 Oscillatory tests

Fruit purees exhibit viscous and elastic behaviour, and therefore the appropriate oscillatory tests were used. Using the MCR at 25°C, using the 25mm serrated parallel plate geometry (25mm diameter, gap height 1mm gap) two amplitude sweeps (10 rad/s) were conducted to determine the linear viscoelastic region (LVR) followed by a frequency sweep in the LVR (0.05% strain, 100 - 0.1 rad/s). A pre-shear was applied prior to each of the above stages for 90s at 10 s^{-1} . This method was used for purees and pelleted materials.

A brief analysis of the CDTA soluble pectins was made using a cone and plate geometry, with a 50mm cone with 0.5° angle, measured at 25°C. An amplitude sweep at 5 rad/s between 0.1 and 1000% strain was conducted to determine the LVR region and then a frequency sweep at 0.5% strain.

3.2.8.3 Choice of geometry

The parallel plate was used for measurement of the purees and pelleted materials described in this report. The inhomogeneous nature of the tomato products containing particles required careful choice of the measuring system, and therefore serration on both plate surfaces were used to counteract wall slip effects. Slip can occur when dispersed systems are in contact with a smooth solid surface; the dispersed phase is displaced from the solid surface, and a thin layer of particle deficient material is sheared along the smooth surfaces. A small gap setting was selected to minimise secondary flow of the serum, and inhomogeneous deformation behaviour. The disadvantage of using the parallel plate over a cone and plate is that the shear rate value at a constant rotational speed is not constant through the entire shear gap, and instead is dependent on the distance from the rotational axis. In addition to this the shear rate in parallel plate measuring system is also dependant on the distance between the plates. However the tomato puree contains particles sufficient in size that, when in motion, would influence the deformation and flow behaviour in a cone and plate measuring system (Mezger, 2006).

The problem of slip has been of concern to other researchers, and has been overcome by using a four-bladed vane geometry to measure dynamic rheological measurements of concentrated tomato pastes, (Bayod, Willers and Tornberg, 2008). However, with use of a vane geometry, secondary flow can occur with lower

viscosity samples such as the ones analysed in this report, therefore the serrated parallel plate was considered the most suitable choice for this work.

3.2.8.4 Stirred viscosity (RVA)

A Rapid visco analyser (RVA) Super 4 (Newport Scientific, Sydney, Australia) was used to obtain stirred viscosity values at 25 °C on 25 g of sample at 180 rpm. The RVA is a mixing system with an impeller (paddle) in a small canister holding the sample, Figure 3-5. It is mainly used for measuring the pasting and gelling behaviour of starch slurries and starch based products such as maize grits (Becker, Hill and Mitchell, 2001) during a heating and cooling regime over a specified time period. A constant shear rate is applied to the sample (controlled shear rate), the resultant torque is measured and viscosity is presented in centipoise (cP), 1 cP is equivalent to 0.001 Pa s. The shear rates involved during stirring are poorly defined because of the complex shape of the geometry (impeller) and the shear rate varies across it. In this study, the RVA was used to determine stirred viscosity data for the puree over a range of total solids. The use of the RVA for determining the viscosity of tomato puree is novel however, it was found to be suitable for quickly determining a viscosity measurement on a relatively small amount of sample at a constant temperature. Lai, Steffe and Ng (2000) have calculated average shear rates of the RVA impeller experimentally, and from this data, the average shear rate used in this work at 180rpm can be assumed to be 60 s^{-1} .

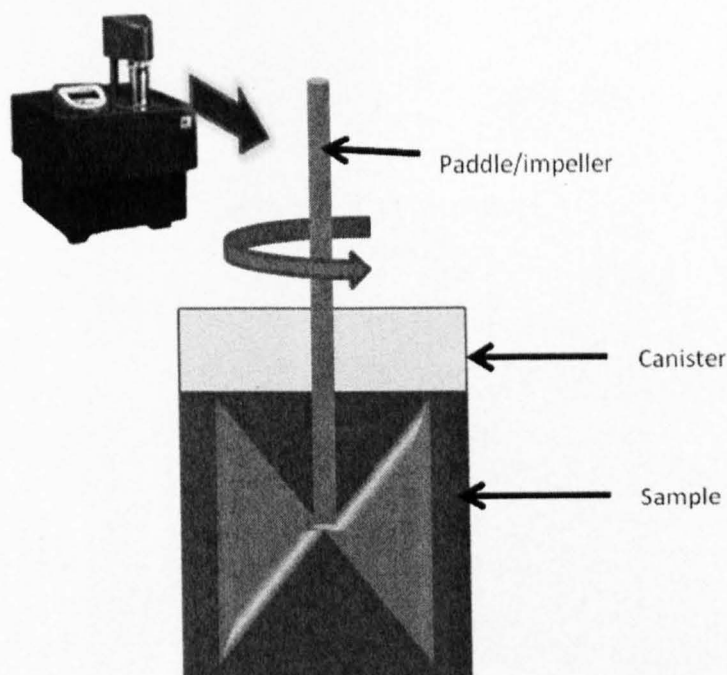


Figure 3-5 Schematic diagram of the RVA rapid visco analyser

3.2.9 Particle size

The particle size distribution of the puree samples was determined using a laser diffraction particle sizer (Beckman Coulter LS-13320, Meritics, Dunstable, UK). Three replicates were taken for each sample. Distilled water was used as the carrying solution. The pump speed was 31% power throughout; and sufficient sample was added to obtain an obscuration value of typically 30-40%. After an equilibration period of 3 minutes, data was collected for 60 s and the diffraction data were analysed using the Fraunhofer diffraction method with the refractive index assumed to be 1.6. Results expressed as equivalent spherical diameter were calculated by the software based on the volume or area of the particles;

$$d_{43} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad \text{Equation 3-1}$$

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad \text{Equation 3-2}$$

where n_i is the percentage of particles with the diameter d_i . The volume based diameter (equation 3-1) is more important for larger particles, whereas the area based diameter (equation 3-2) takes into greater account the smaller particles, which are thought to be important for filling the voids between larger particles and thus affect viscosity and create a more continuous network of particles in the suspension.

3.2.10 Microscopy

The light microscope (Leitz diaphan) was used to obtain images of one drop of the tomato puree at x4 magnification. A cover slip was used.

3.2.11 Particle volume and weight measurements by centrifugation

Separation of the insoluble particles from the serum is achieved by gravity when centrifuging puree. The rate of sedimentation is due to factors such as; viscosity of the liquid phase, particle density and particle size, concentration of the solution (fraction of dissolved solids) and the force of gravity. The centrifugation process can damage the fragile particles and cause liquid to diffuse from the particles, leading to inaccurate determination of the volume fraction. However, despite these drawbacks, the method can be used to gain useful information about the particle volume (for comparison with the Krieger Dougherty Equation 2-3), because the insoluble particles and soluble fractions can be separated and (relatively) independently analysed to determine the contribution of each to the overall viscous and elastic properties of the puree.

3.2.11.1 Assessing the effect of force and time

To assess the most suitable centrifugation conditions to achieve compaction of the swollen materials where the volume of the pellet did not alter with centrifugation time, samples of Sainsbury's tomato puree were centrifuged at 1000-4000 g for between 10-80 minutes and the volume and weight fraction of the particles calculated. Samples were done in triplicate. From this experiment, the most suitable conditions were selected as 2700g for 60 minutes. These conditions were used for all other centrifugation experiments.

3.2.11.2 To separate the particles from the serum

After the puree samples had been centrifuged, the weight and volume of the total sample were noted and then pelleted material containing the particles were separated from the serum. The serum (supernatant) was carefully poured out from the centrifuge tube and the tube inverted for 30 minutes. The pellet was weighed and the ϕ volume of the particles calculated.

3.2.12 Total solids and Brix

The total solids of the puree samples were determined by oven drying for 18 hours at 105°C. Brix was measured using an Atago PAL-1 digital pocket refractometer (Atago, Tokyo, Japan). The principle of Brix measurement is that the density of a substance increases (due to increased soluble solids) and the refractive index increasing proportionately. The Brix scale is based on a sucrose and water solution.

However, since tomato contains substances other than sugar (salts, minerals) the Brix percentage represents the total concentration of all soluble solids in the sample.

3.2.13 Statistical analyses

3.2.13.1 Commercial tomatoes

A one way ANOVA using Design Expert (Version 6.011 Stat-Ease, Inc Minneapolis, USA) was used for the statistical analysis for the commercial tomatoes.

3.2.13.2 Introgression lines

ANOVA (analysis of variance)

A one way ANOVA was done on the results to determine if there were statistical differences between the IL lines ($P < 0.05$), then Tukey's multiple comparison test with a confidence interval of 95% was used to determine how the tomatoes from the IL lines (categories) were grouped.

Tukey's test

Tukey's test is a single-step multiple comparison statistical test. It was used in conjunction with the ANOVA to find which of the mean values were significantly different from one another. The Tukey's test compared all possible pairs of means, and is based on a studentised range distribution (this distribution is similar to the

distribution of t from the t -test). The test compares the means of every IL to the means of every other IL; that is, it applies simultaneously to the set of all pairwise comparison and identifies where the difference between two means is greater than the standard error would be expected to allow. The assumptions of the test are that the observations being tested were independent, and that there was an equal variation across observations.

Test for the significance of the Pearson product-moment correlation coefficient

The relationship between some parameters, for example between serum total solids (x-axis) and Brix (y-axis) in Figure 7-13, was tested by firstly establishing their linear correlation, The Pearson product-moment correlation (R), and the coefficient of determination (R^2). Both R and R^2 were calculated using Microsoft Excel. (Redmond, Washington: Microsoft, 2010. Computer Software).

The hypothesis that the relationship between the two parameters occurs by chance and shows a positive or negative correlation can then be tested. If the true correlation between x and y within the general population is $R=0$, and if the sample size is N and greater than 6, then a t value can be calculated, as shown in the equation below. This t value can be used to calculate the probability that the value occurred by chance. Significant correlations were said to have occurred if the t or p value was ≤ 0.05 .

$$t = \frac{R}{\sqrt{(1-R^2)/(N-2)}}$$

Equation 3-3

Multivariate principle component analysis

Multivariate principal component analysis (PCA) (Unscrambler, Camo Software, Oslo, Norway, release 9.0) was used for statistical analysis on the assessment data for the viscosity screen of the introgression lines. This was done to obtain an overview of the way in which the different physical properties of the tomato purees were interrelated.

3.2.13.3 Comparison of texture and viscosity of the control (M82) with IL3-4, an IL producing firm textured fruit

A two way ANOVA was done on the results to determine if there were statistical differences between the control M82 and the 'firm fruited' IL3-4. ($P < 0.05$), factor 1 was colour (numerical) and factor 2 was the IL line (category).

CHAPTER 4: Colour and Texture

4 Colour and texture

4.1 INTRODUCTION

Part of the work in this dissertation was designed to test the hypothesis that the texture of tomato fruit correlates with the viscosity of the puree, see hypothesis (i) in section 2.6. To establish methods and values to quantify whole tomatoes and to help identify the general stage of ripeness (see Figure 2-15), a series of work was undertaken to look at tomatoes harvested at different ripeness, defined by the colour of the fruit. The factors investigated for these samples were their colour, texture, solids content, soluble solids and pH.

4.2 RESULTS AND DISCUSSION

The tomatoes used were from a commercial supplier and sorted by into four types by colour by hand. They were tested for colour and texture within one day of delivery and stored at 4°C until these measurements could be done. The remaining tomatoes from the four types were deseeded and stored at -80°C until required for viscosity measurement.

The colour of the tomatoes were assessed Table 4-1, using the Minolta Chroma meter CR-400 (Konica Minolta) with the a* chromaticity coordinate, with values

negative to positive expressing the colour change from green to red, the protocols as described in the methods section 3.2.4. The colour changes indicated the key stages of tomato ripeness, as defined by the a^* values of the surface of the tomatoes, Table 4-1, and the four categories of tomatoes were significantly different one from the other ($P \geq 0.05$) in terms of colour, and maximum load values). A photograph is shown in Figure 4-1 with examples of fruit from the four observed colours and guided by Figure 2-15 they were classified into the four stages of maturity; mature green, breaker, pink and red ripe.



Figure 4-1 Photograph of the tomatoes typical of the four observed stages of ripeness, defined mature green, breaker, pink and red ripe, left to right. The black line represents 5cm.

Measures of texture will be dependent on how the fruit is presented to the probe during measurement. Section 2.2 in the literature review explains the different areas of the tomato. As it could be expected, gross changes in texture could vary depending on the location, two areas to probe for texture were chosen, the outer and inner pericarp. The method used for texture assessment is given in section 3.2.4.

Table 4-1 shows that as the tomatoes ripen, mean maximum load decreases, and the a^* value increases. This was as expected; fruits softened as they turned from

green to red. The mature green tomatoes (mean a* value -10.3) had higher maximum load values and therefore had firmer inner and outer pericarp texture than the red ripe tomatoes (highest positive a*value, 19.8). The relationship between the softening of flesh and colour change of the flesh was expected, and has been demonstrated in many studies such as Powell et al (2003), and is shown in Figure 2-15. Measurement values show that the fruits softened from the inside to the outer part of the fruit, the inner pericarp decreased from 2.3 N at the mature green stage, to 0.5 N at the pink stage, compared with the outer pericarp going from 2.3N to 0.9N at the same stage.

Table 4-1 Mean values and (standard deviations) of the ten replicates of texture and colour analysis on tomatoes of different stages of ripeness.

Ripeness stage	Colour	Maximum load N	
	a* mean value	Outer pericarp mean value	Inner pericarp mean value
Mature green	-10.3 (1)	2.3 (0.3)	2.3 (0.2)
Breaker	-3.2 (2.6)	1.7 (0.1)	1.3 (0.2)
Pink	7.6 (1.9)	0.9 (0.4)	0.5 (0.1)
Red ripe	19.8 (2.6)	0.5 (0.1)	0.4 (0.1)

Table 4-2 Total solids, Brix and pH of hot break puree made from tomato pericarp tissue, and total solids and cell wall material (AIS) content of raw pericarp, at four stages of ripeness

	Raw pericarp		Hot break puree		
Ripeness	Total solids %	Cell wall material (AIS) %	Total solids%	Brix %	pH
Mature green	4.5 (0.10)	1.49 (0.05)	5.4 (0.09)	4.3	4.4
Breaker	4.5 (0.11)	1.1 (0.01)	5.7 (0.01)	5.0	4.5
Pink	4.3 (0.01)	Not tested	5.0 (0.6)	4.5	4.2
Red ripe	4.6 (0.04)	0.63 (0.02)	5.7 (0.05)	5.5	4.0

Table 4-2, shows that both raw tomato and the puree processed in the laboratory were highly hydrated at approximately 95% moisture, and as the fruit ripened, the AIS (alcohol insoluble solids, defined as the cell wall material) content of the pericarp decreased from 1.49% to 0.63% from mature green to red ripe. A point to note was the fact that the total solids content of the raw pericarp and the puree did not show a clear trend with ripeness, suggesting that the differences in texture of the pericarp tissue was not related to the amount of solids present.

The puree was made as explained in section 3.2.6.1. During the processing of the puree from the raw tomato pericarp, some evaporative losses occurred during the heating stage; the puree had approximately 1 % higher total solids than the raw pericarp. The approximate 5% solids in the raw pericarp and the purees was a combination of water insoluble soluble and water soluble solids. The water soluble solids can be defined as Brix. Brix was measured in this study according to the method given in section 3.2.12. As the tomato ripens, the total solids and Brix of the puree varied within the range of 5-5.7% and 4.3-5.5% respectively. At the red ripe stage, it is likely the solids have been broken down into smaller molecules, shown by the water soluble solids increasing to 5.5% Brix, (and the AIS decreased in the raw pericarp).

4.3 CONCLUSIONS

This chapter has started to test hypothesis (i) of the thesis (see section 2.6), that texture and ripening may relate to the viscosity of the pureed fruit. Tomatoes of different stages of ripeness, as shown by differences in their colour have been sourced, and analysed. Analysis showed that the most important change in ripeness observed was the increase in Brix and decrease in AIS (cell wall material). Since the ripening process is associated with changes in the overall composition of the solids to an increase in soluble solids (in the literature review section 2.3 ripening is discussed in association with dissolution of the middle lamella and structural changes in the networks of cellulose, cross-linking glycans (hemicelluloses), and pectins in the cell wall (Brummell, 2006)); the question arises whether the ripening and softening of the fruits is related to quality of the solid material in terms of structuring and viscosity. Therefore hypotheses (ii) and (iii), (stated in section 2.6) are to be tested in the next chapter. The protocols for measuring the rheology and preparation of the puree are required. The methodology for such preparation and measurement are discussed in the next chapter (chapter 5), ready for the main assessment of the puree from the four stages of ripeness, as presented in chapter 6.

CHAPTER 5: Rheological properties of puree

5 Rheological properties of puree –assessing methodology and preparation

5.1 INTRODUCTION

The previous chapter established that a change in colour and texture occurred as the fruit ripened; the fruit develops the characteristic red ripe colour as well as the flesh softening. The aims of this chapter were to establish preparation and measurement protocols suitable for the quantitative characterisation of the mechanical properties of tomato puree so that different samples can be compared with clarity. Hypotheses (ii) and (iii), (see section 2.6) require precise and practical measurement of the rheological properties of puree. As such, this chapter considers aspects of aims (ii) and (iii) of the thesis, outlined in section 2.6. In the next chapter (chapter 6) it will be investigated whether the ripening-related change in texture and solids composition affects the viscosity of the fruit once the flesh has been broken down into puree.

Puree is a mix of particles and a serum of soluble solids in solution (including polymeric and simple sugars). Microscope images of this complex microstructure are shown in Figure 5-1 as examples. For measuring the individual contribution of the two phases to puree rheology, the methodology of separating the insoluble particles from the serum is also a critical factor. The method of separating the

insoluble particles and serum using centrifugation was assessed in terms of the effect of the centrifugal force and duration of centrifugation on the relative amounts of the particle and serum fractions. This was considered important as the particles are hydrated by the serum fraction. Only after separation was it possible to assess the rheology of the insoluble particles and the serum independently.

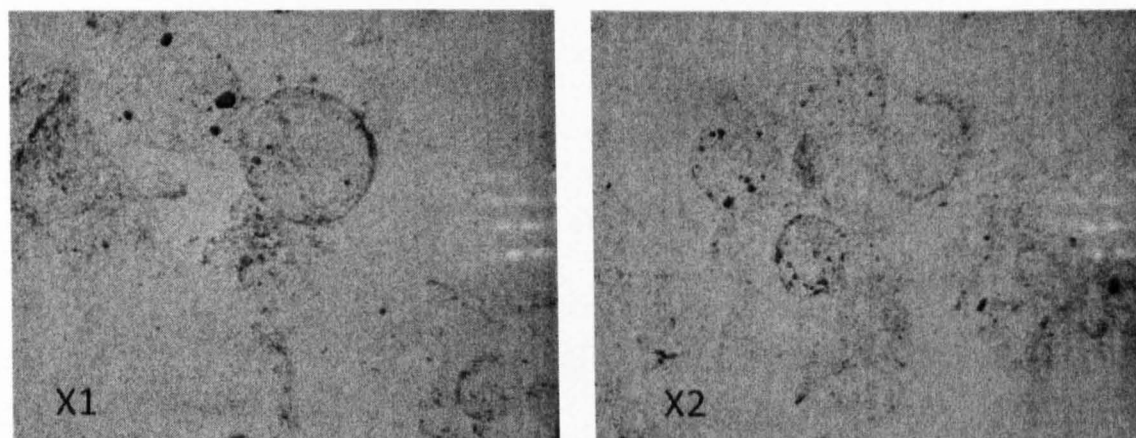


Figure 5-1 Image of Sainsbury's tomato puree, batches X1 and X2 as viewed under the light microscope. Scale bars 200 microns.

In view of the above limitations and challenges in measurement, a range of measurements were developed, using a ready prepared puree (see materials section 3.1.1 for details), to establish protocols of puree measurement to ensure a reproducible method for determining the rheological properties. The results of these protocols on ready prepared puree are presented and discussed in sections 5.2.1 and 5.2.2, and then later in section 5.2.3 the protocols for preparation of puree prepared from the tomatoes of different stages of ripeness will be presented.

5.2 RESULTS AND DISCUSSION

5.2.1 Fundamental rheology

Tomato based products such as puree exhibit viscoelastic behaviour, as can be observed in Figure 5-2, typical of suspensions; i.e. both viscous liquid (G'') and solid elastic (G') behaviour over a range of conditions. Finding the extent of the linear viscoelastic behaviour was the first test to be conducted on the commercial puree, in the form of a strain sweep, 0.01-100% strain, at an angular frequency of 10 rad/s, Figure 5-2 . The linear viscoelastic domain extended to approximately 1% strain. G' (storage modulus) values were larger than G'' (loss modulus) for the puree which was an indication of the elastic nature of the material predominating under these conditions. Each sample was pre-sheared at 10 s⁻¹ for 90s before each rheological test (i.e. the sample was sheared for 90s before each strain sweep, and again before the frequency sweep was done). Amplitude sweeps were superimposable one upon the other on two consecutive runs this indicates that the measurement does not impose any further permanent changes in the system, for example the particles are not being broken down in the regimes used and that any time dependent changes are disrupted in the pre-treatment.

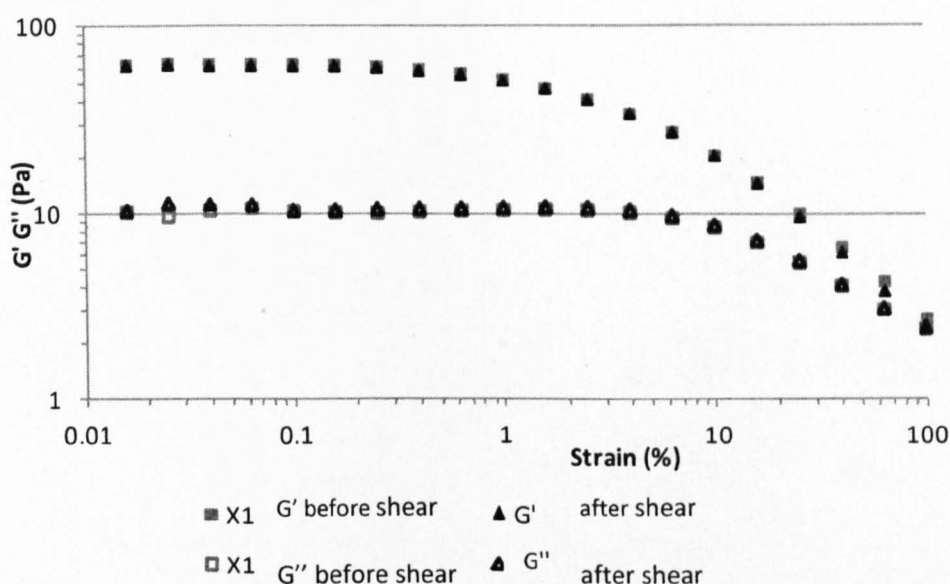


Figure 5-2 Strain (amplitude) sweep performed at 10 rad/s Sainsbury's puree batch X1. The elastic/storage modulus G' (filled symbols), and the loss modulus G'' (open symbols), before and after a shear interval of 90 s at 10 s^{-1} .

Based on the strain sweep experiments, a strain of 0.05% was used for the frequency sweep (Figure 5-3), so that it was conducted within the LVR. G' and G'' were shown to be independent of the angular frequency (from 0.1- approximately 10 rad/s), indicating a gel like behaviour. In fact, the behaviour could be described as a *weak* gel-like behaviour, with G' higher than G'' at all the frequencies, with G'' lower than G' by less than 10-fold. The G' at 10rad/s corresponds to the G' plateaux value measured in the amplitude sweep at 0.05% strain, showing good agreement of the data and also indicating that the measurements are not altering the matrix.

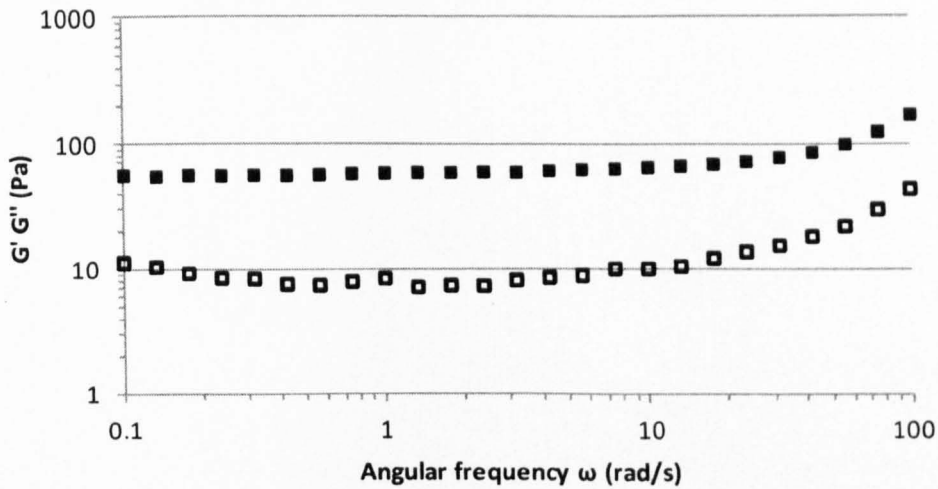


Figure 5-3 Frequency sweep at 0.05% Strain Sainsbury's puree batch X1, G' represented by the filled symbols, G'' with open symbols.

Measuring the viscosity over a range of shear rates showed that Sainsbury's tomato puree was a shear thinning material, Figure 5-4 with no shear dependent structure observed under the shear conditions used, as indicated by the down (high shear rates to low) curve showing similar viscosity values to the up curve for the tomato puree tested. The shear thinning nature of the puree fitted a power law model relatively well, Figure 5-5 with an R^2 value of above 0.9. The consistency index, K was calculated as 5.14 and the flow behaviour index (n in equation 2-4) was 0.141 indicating a shear thinning behaviour.

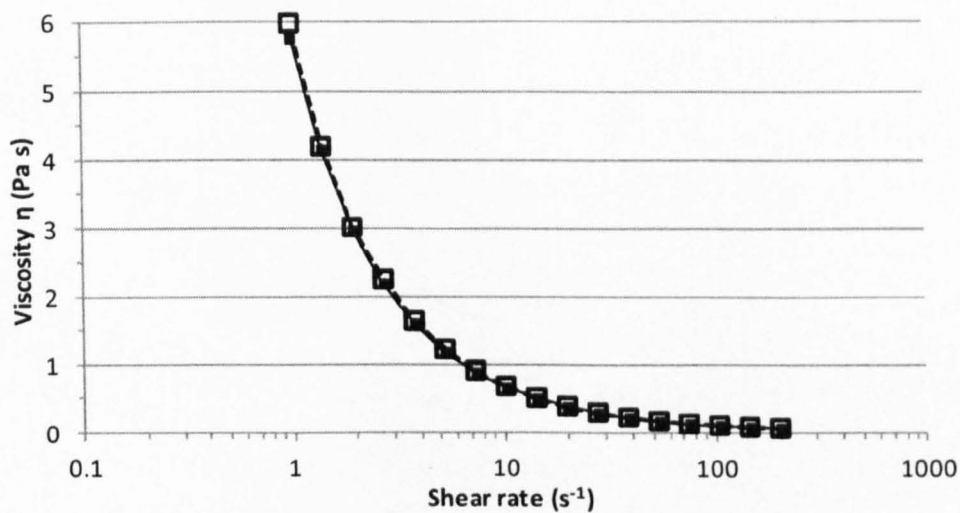


Figure 5-4 Flow curve of Sainsbury tomato puree, X1. Filled symbols – 1-200 s^{-1} , open symbols 200-1 s^{-1}

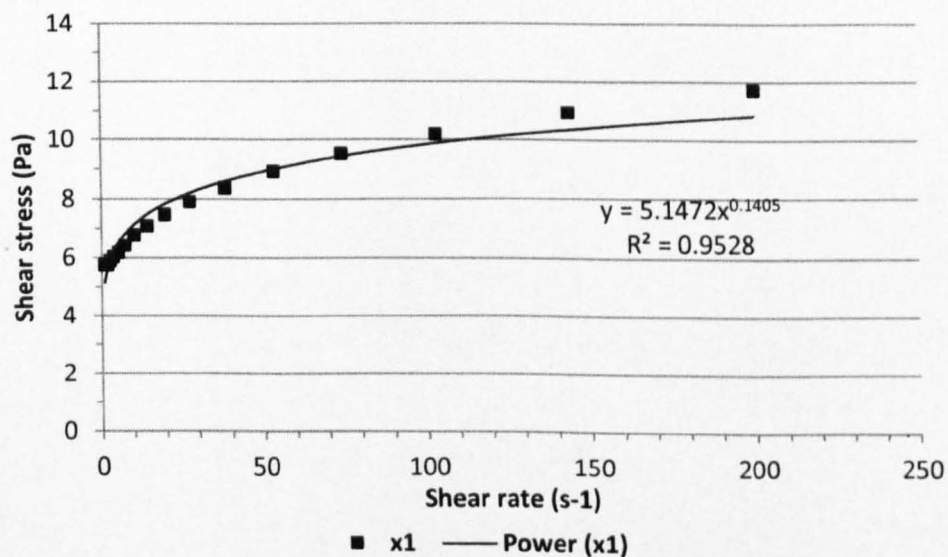


Figure 5-5 Flow curve of Sainsbury tomato puree X1, measured between 1-200 s^{-1} and power law.

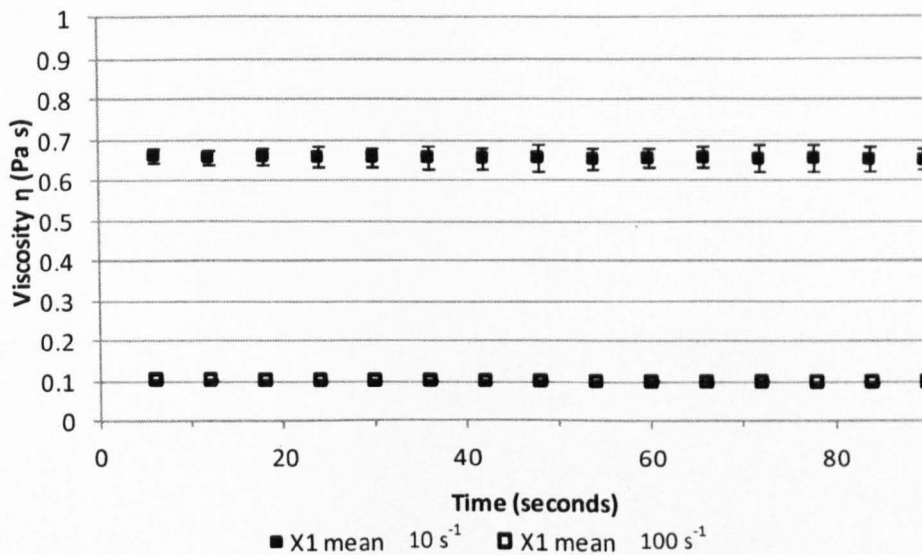


Figure 5-6 Viscosity at 10 and 100 s⁻¹ over 90 second measurement time for Sainsbury puree. Error bars represent standard deviations for three replicates (gap fillings)

To check the repeatability of these rheological measurements, three replicates, i.e. three gap fillings of these measurements were tested on X1 and X2 puree samples. Figure 5-6 shows as an example the apparent viscosity at 10 and 100 s⁻¹ for the Sainsbury's tomato puree. The standard deviation for the three reps was less than 5% for both shear rates measured. The conclusion that could be drawn from this is that the repeatability is relatively good.

The final comparison on the puree as a whole was to determine if there were any differences between batches of the commercially prepared tomato puree. In order to do this, two batches of Sainsbury's puree defined as X1 and X2 were tested using the MCR rheometer. As shown in Figure 5-7, there was a statistical difference between apparent viscosity values of the two batches, indicated by narrow standard deviation error bars and a large difference in the mean apparent viscosity

values of 0.66 and 0.87 Pa s at 10 s^{-1} for Sainsbury carton puree batches X1 and X2 respectively. This finding was important for two reasons; that even commercially prepared puree could vary in viscosity and the methodology was sufficiently sensitive for this difference to be observed.

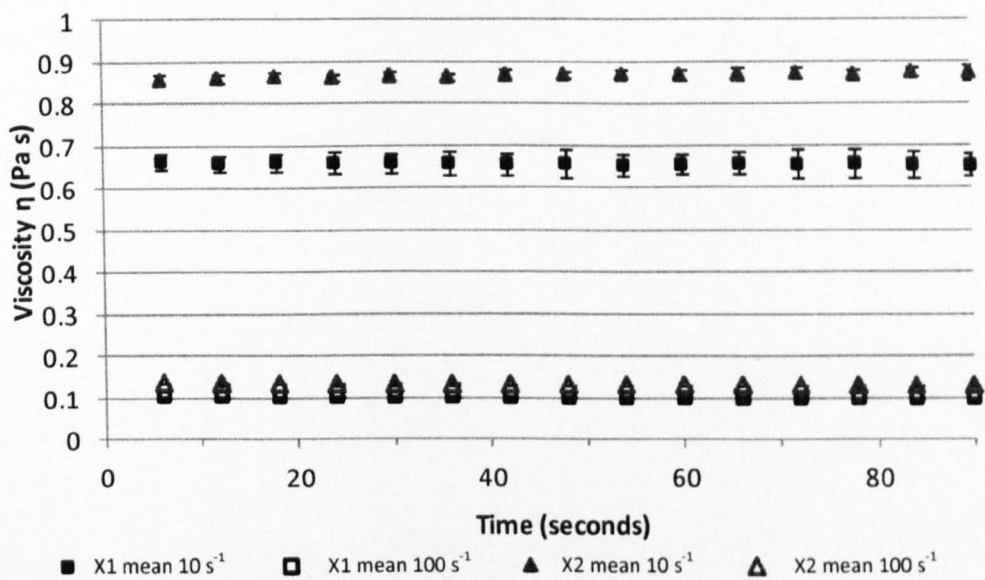


Figure 5-7 Viscosity at 10 and 100 s^{-1} over 90 second measurement time for two batches of Sainsbury puree, X1 and X2. Error bars represent standard deviations for three replicates (gap fillings)

5.2.2 Empirical measurements of viscosity –stirred viscosity

The RVA (rapid visco analyser) was used alongside the more fundamental rheology detailed above. This was to determine an empirical measurement of viscosity, or stirred viscosity for the tomato puree samples, which could be important due to the relatively large particles in tomato puree samples. The RVA method is detailed in section 3.2.8.4.

The aim of these preliminary experiments was to determine the repeatability of the RVA in measuring the tomato samples. In the experiments explained in this chapter,

the tomato samples were stirred at 180 rpm for 10 minutes at 25°C, and measurement points taken every eight seconds, examples of typical RVA data for two puree samples are shown in Figure 5-8.

The mean viscosity of three replicates of pre-prepared puree (Sainsbury's carton), X1 and X2 over the 10 minute analysis period was 108 and 132 cP, respectively and values are shown in Figure 5-8. The small standard deviation between replicates shows that the RVA produced repeatable measurements and had a 2% coefficient of variation. As in the more fundamental measurement of viscosity using the parallel plate, Figure 5-7, X2 Sainsbury's puree/puree had a higher viscosity than X1.

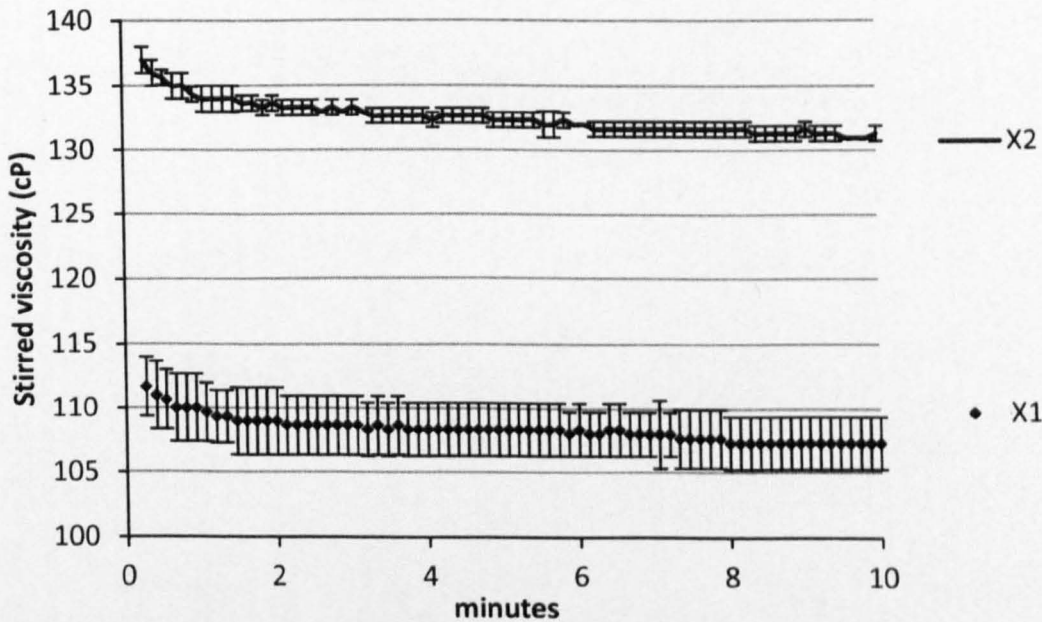


Figure 5-8 RVA stirred viscosity for Sainsbury's carton tomato puree measured at 180rpm at 25°C. [The figure shows the difference in stirred viscosity between two batches, X1 (below) X2 (above). The mean values of three replicates are shown with error bars representing the standard deviation]

The mean shear rates for samples stirred in the RVA have been calculated and at 180rpm is equivalent to $60s^{-1}$ (Lai et al, 2000). Therefore the stirred viscosity value

obtained using the RVA, Figure 5-8, and the apparent viscosity calculated from a flow curve measured using the MCR can be compared at a common shear rate. The values obtained are shown in Table 5-1 and show apparent viscosity values of 0.15 and 0.20 Pa s Sainsbury's puree batches X1 and X2, respectively. These values were considerably higher than stirred viscosity values obtained using the RVA. The assumption that the RVA impeller average shear rate was 60s^{-1} may be misleading as the range of shear rates across the paddle are large. At the tips of the RVA paddles, where the higher shear are found, it is possible that the particles are broken, but there is not a very large effect as the decline in viscosity with time as Figure 5-8 indicates. This comparison of the methods shows that it is difficult to utilise different rheological testing regime and achieve the same viscosity values.

Comparisons between dynamic viscosity and viscosity from flow curves can also be done by applying the Cox-Merz rule, (Cox and Merz 1958), (see section 2.5.2.2 of the literature review for an explanation of this semi-empirical rule). This is shown in Figure 5-9, where the complex and shear viscosities for tomato puree were plotted on the same chart. This figure shows that tomato puree viscosity behaviour deviates from the Cox-Merz rule; this has been noted before for tomato pastes and other materials that can be considered dispersions.

Table 5-1 Comparison of viscosity values of the 2 batches of Sainsbury’s tomato puree, measured using the rheometer with a clearly defined gap size, and the RVA, an empirical measurement of viscosity

Sainsbury tomato puree batch	Rheometer (MCR) Apparent viscosity at 60 s ⁻¹ (Pa s)	RVA Stirred viscosity at 180rpm Calculated average shear rate of 60 s ⁻¹ (Pa s)
X1	0.152	0.11 Pa s108cP
X2	0.202	0.13Pa s133cP

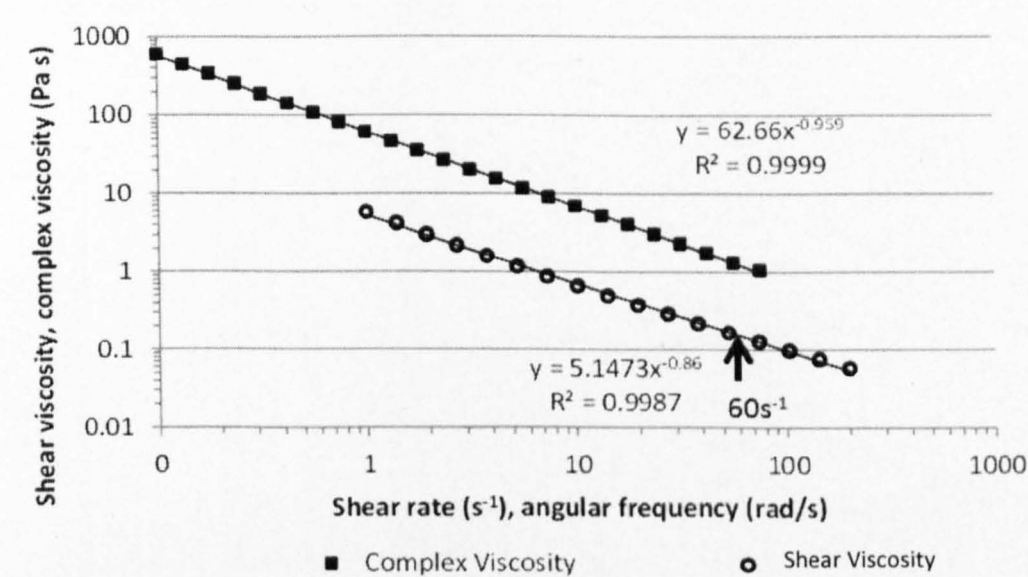


Figure 5-9 Comparison of shear and complex viscosity of Sainsbury’s tomato puree batch X1, with deviation from the Cox-Merz rule.

[An arrow points to the shear viscosity at 60s⁻¹, for comparison with the assumed impeller shear rate used in the RVA measurements]

The conclusion from the differences in viscosity obtained using different measures (RVA and MCR) was that for the complex system of the tomato pastes the rheological measurements were not truly fundamental, but showed good repeatability.

The advantages of good repeatability and ease of use therefore deemed it appropriate to use the RVA for further studies. Concentration would of course make a major difference to the measured viscosity; therefore samples would only be compared at equal concentrations. Conclusions can be drawn from above studies using ready-prepared tomato puree that suitable method of analysis had been developed.

5.2.2.1 Measurement of volume and weight fraction of the particles

The method of separating the insoluble particles and serum using centrifugation was assessed next. It has been reported previously that that pellet weight is an important factor in viscosity of tomato puree (Tanglertpaibul and Rao, 1987), and therefore the measurement conditions of this parameter is considered in this section.

The volume and weight ratio of the particles can be determined by calculating the volume of the pellet and supernatant after centrifugation, as described in the methods section 3.2.11. The dependence of particle volume fraction (pellet to sample volume ratio) on centrifugal force and time is shown in Figure 5-10, and these results indicate that the particles appear to be soft, compressible and possibly lose volume due to exclusion of the internal fluid, as the volume fraction decreases within the range tested (1000-4000 g and 10-80 minutes). Thus, indicating that compression of the particles had occurred, 'squashing' some of the liquid phase from the highly hydrated particles. It is also possible that air could be entrained in

some samples and this may be displaced by centrifugation. A similar observation was made in previous work by (Lopez-Sanchez et al. 2012) on shear elastic deformation and particle packing in plant cell dispersions and their paper shows microscope images of tomato particles before and after centrifugation showing clearly that deformation of the particles had occurred with the experimental conditions they used.

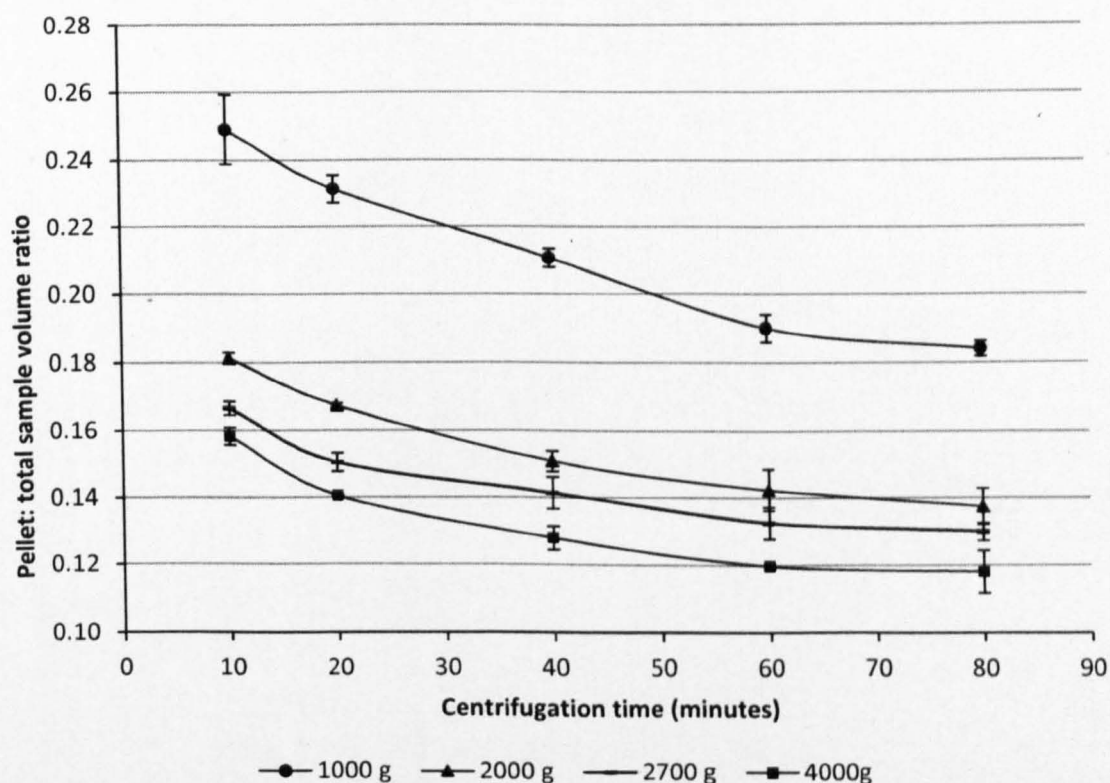


Figure 5-10 The effect of centrifugation speed and duration on pellet to sample volume ratio commercial tomato puree. Values are means \pm one SD of three samples

For all future experiments, 2700 g for 60 minutes was used and these were considered suitable conditions to obtain pelleted insoluble solids for future measurements. The pellet obtained under these conditions were resuspended with sucrose solution (8.4% w/w) at 20, 60 and 80% pellet by weight and used to investigate the effect of pellet total solids on the phase volume by re-centrifuging at

2700g for 60 minutes, Figure 5-11. The increase in phase volume with initial pellet weight was not linear, and the pellet appeared more compressed with the addition of the higher percentage of pellet weight before centrifugation, indicated by a volume fraction ratio below 0.7 when the initial weight of 80% pellet was used. This was a similar finding to Day et al (2010) with a study on plant cell wall particles; they postulated that the particles being deformed through over-packing, or not fully swelled as a result of restricted space during rehydration. Since the pellet ‘lost’ weigh after the re-suspension and subsequent centrifugation, it also indicates that some of the water soluble material may have moved into the serum phase.

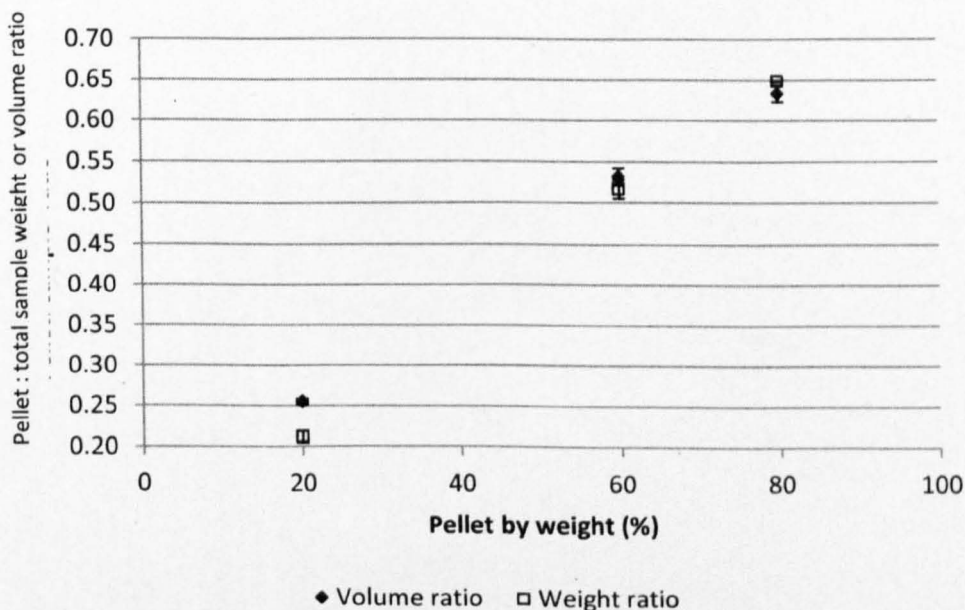


Figure 5-11 Effect of pellet concentration on the volume and weight ratio. Mean values shown of three replicates, with error bars representing the standard deviation

The pellet volume and weight ratios were determined for the two batches of commercial tomato puree, Table 5-2 and compared with the apparent viscosity values obtained in section 5.2.1. Batch X2 puree was shown to have the highest apparent viscosity in Figure 5-7 also has the highest pellet volume and weight ratio. The importance of particle volume fraction is a concept discussed further in the

next chapter (chapter 6) with reference to the deformability of the particles from fruit at different stages of maturity, and chapter 7 for the introgression lines at the red ripe (B+7) stage.

Table 5-2 Volume and weigh ratio of commercial tomato puree samples X1 and X2

	X1	X2
Pellet volume ratio	0.16	0.20
Pellet weight ratio	0.14	0.17

5.2.3 Preparation of the laboratory prepared tomato puree

The processing method to prepare the puree from tomato may have a significant effect on the viscosity of the tomato puree, as discussed in the methods chapter 3.

The major concerns for the process and analysis included:

- i. Heat treatment of the tomato
- ii. Storage of the puree
- iii. Effect of stirring on laboratory prepared tomato puree
- iv. Ensuring the methods developed were sensitive enough to determine differences between samples.

Therefore the next set of experiments was done to develop a reproducible method of preparing the tomato pericarp into puree, so that in later experiments texture, ripeness and viscosity of puree could be compared in future experiments (see Figure 6-1 for results).

5.2.3.1 *Heat treatment of the tomato*

The method of preparation was tested on puree made from red ripe tomatoes initially whilst the effect of a heat treatment (called 'hot break') was investigated. The hot break process is commonly used in commercial tomato puree manufacture and involves a period of rapid heating to inactivate enzymes, such as polygalacturonase, shown to be involved in the softening of fruit during ripening. A hot break process was chosen to inactivate these enzymes and was compared with a cold break, whereby no heat treatment is used. Theoretically it was felt this was an important aspect of the experiment to decide which was best because on one hand the energy input due to the heat treatment could affect the structures of the tomato tissue; on the other hand inactivation of the enzymes would perhaps

'preserve' the structure so the tissue texture and viscosity of the pureed tissue could be compared more accurately.

Frozen tomato pericarps were peeled by hand and cut into 1cm cubes. For the hot break process, the tomato was heated to $95\pm 2^{\circ}\text{C}$ in the microwave. The cold break sample was left for 12 hours at 4°C to defrost. Both hot and cold break tomatoes were then blended for the same length of time using a hand held blender and afterwards viscosity measurements were determined using the RVA. Three samples of tomato pericarp were separately prepared for the hot and cold break preparations were made (independent replicates). These preparations were adjusted, by the addition of water, if necessary to give the same solids content. As shown in Figure 5-12, the hot break process used before the pureeing step produced a higher mean viscosity (140cP) than the cold break method (110cP). The smaller standard deviation shows also that the hot break process was more repeatable, i.e. had less variation (indicated by the smaller standard deviation). Figure 5-13 shows images of both the hot and cold break microstructure as viewed using a light microscope, and from initial observations the microstructure did not differ greatly. From these initial results, a hot break method was used for all future preparations.

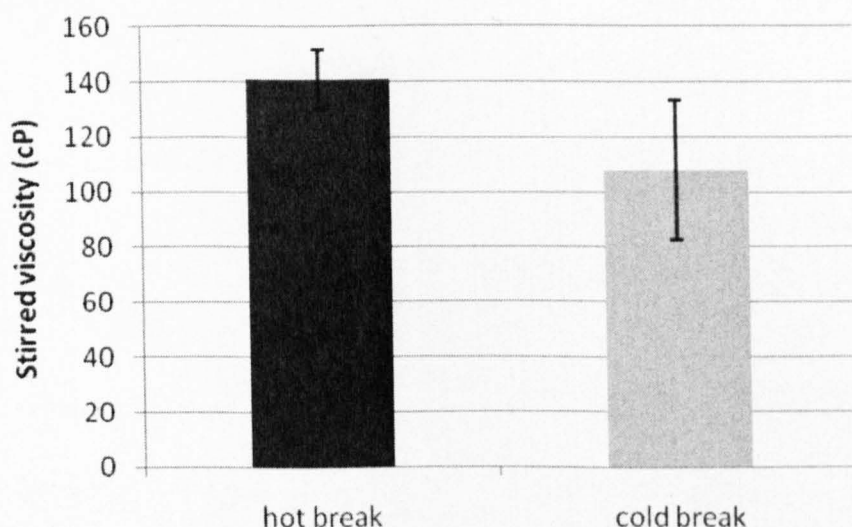


Figure 5-12 Mean viscosity of ripe supermarket tomatoes, hot break puree heated to 95°C before blending, cold break left at 4°C overnight before pureeing. Samples diluted to 3.5% TS before analysis t-test carried out. Significant difference at ($p < 1\%$) between the mean viscosity of hot break and cold break tomato purees

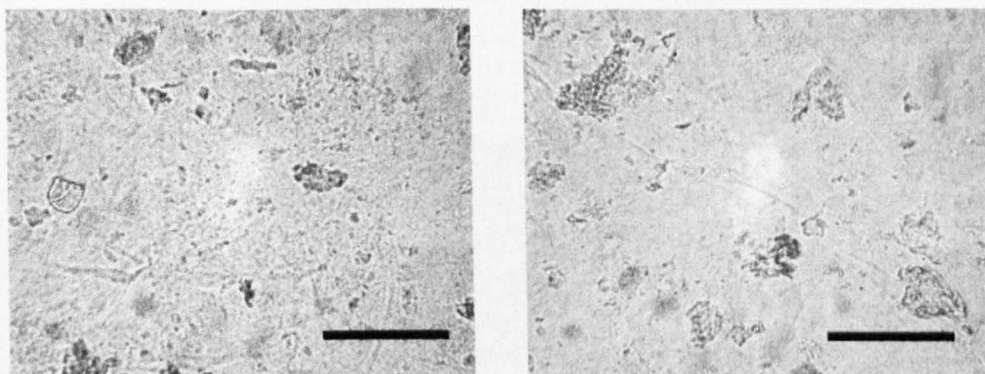


Figure 5-13 Light microscopy images of cold break (left) and hot break (right) puree. Scale bars 50µm

5.2.3.2 Effect of storage time on viscosity of laboratory prepared hot break puree

It was important to determine the stability of laboratory prepared tomato puree so that the rheological tests could be done without concern of storage time affecting results. To test this, the viscosity of a hot break red ripe tomato puree was measured at intervals over a week, with samples stored at 4°C. The tomato puree was prepared as described in the methods chapter section 3.2.6.1 and the time

counted from the end of the blending process. The mean viscosity over the 10 minute stirring time in the RVA was plotted for each measurement made during the week, Figure 5-14. These were the results of the same sample, re-measured over the week. This showed the viscosity decreased by 26% from 199 to 147cP within the first 24 hours. Between 1 and 7 days the viscosity remained relatively stable. This indicated that the method used to prepare the puree created a relatively stable sample; chemical/enzymic degradation occurred within the 24 hours after the preparation step (some, but not all of the initial decrease in viscosity was attributed to the effect of shear). Therefore all subsequent measurements were done on samples the day after they were prepared.

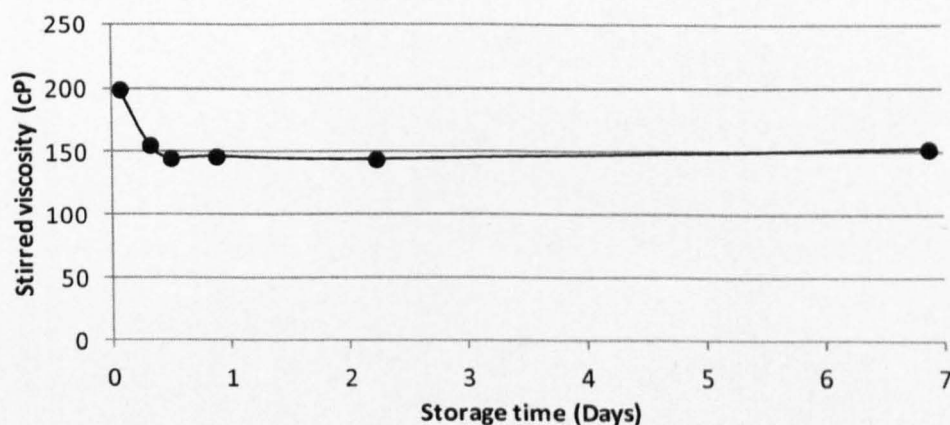


Figure 5-14 Effect of storage time on laboratory prepared, hot break red ripe tomato puree viscosity.

5.2.3.3 Effect of shear on stirred viscosity

It was observed in the previous experiment that the viscosity decreased within the first stirring period with the RVA paddle. The effect of prolonged stirring on the laboratory prepared (hot break) red ripe tomato puree was therefore determined by stirring a sample for 30 minutes at 180 rpm at 25°C in the RVA, Figure 5-15. At the beginning of the measurement period, the stirred viscosity for ripe tomato

puree was relatively high at 251cP, and this decreased rapidly within 10 minutes. After 30 minutes stirring, viscosity had decreased to 162cP. In other words, the red ripe puree lost over 30% of its initial viscosity after 30 minutes. Particle size (see methods section 3.2.9) was measured before and after the stirring process, shown in Figure 5-16. After the stirring process, some of the larger particles in the red ripe puree had been broken into smaller particles, as shown by the particle size measured before and after being subjected to shear in the RVA. This was interpreted as partial break-up of the microstructure due to the mechanical energy of stirring.

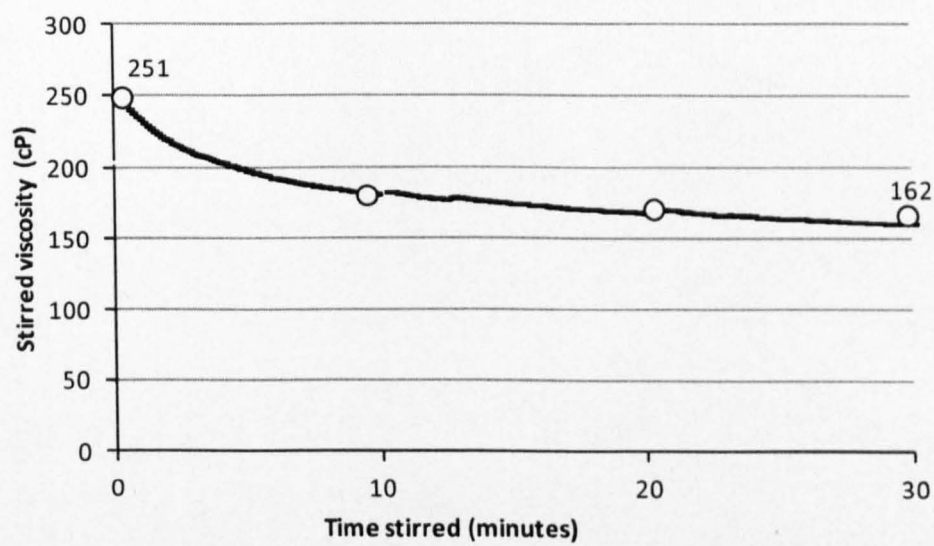


Figure 5-15 Stirred viscosity of laboratory prepared red ripe tomato puree. Effect of prolonged stirring in the RVA

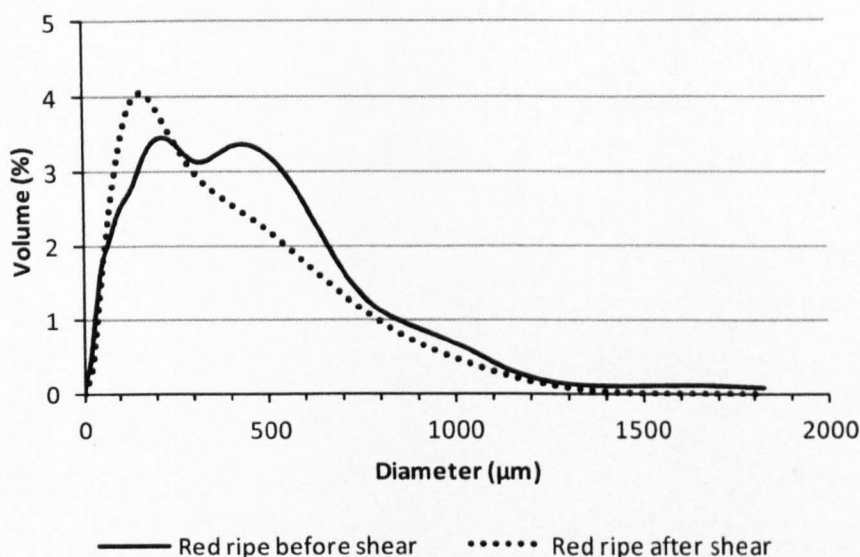


Figure 5-16 Particle size of laboratory prepared red ripe tomato puree before shear and after shear (RVA 180rpm 10 minutes)

5.3 CONCLUSIONS

In this chapter, the methodology for preparation of puree and rheological measurement have been established and tested, aim (ii) in section 2.6, and understanding of the features of the rheology of pureed materials, aim (iii) in section 2.6), have been investigated.

The work described in this chapter has shown that the viscoelastic properties of ready prepared Sainsbury's tomato puree could be determined with good repeatability using fundamental rheological techniques using the Anton Paar MCR rheometer. For example the apparent viscosity at 10 and 100 s^{-1} for the Sainsbury's tomato puree samples X1 and X2, the standard deviation for the three replicate measurements was less than 5% for both shear rates measured. The RVA data showed that the RVA could be used to obtain stirred viscosity values with good repeatability in terms of replicate measurements of the Sainsbury's puree, and gave comparable results with the apparent viscosity calculated from the MCR data,

although the RVA stirred viscosity values were consistently lower than when measured in the rheometer. One of the main hypotheses of this thesis was that a simple stirred viscosity test could be used on tomato puree so that comparisons could be made between different samples (see hypothesis (iii), section 2.6. These findings and conclusions confirm that the RVA can be used for that purpose. Using the RVA and rheometer combined has provided an accurate way of measuring the viscous and elastic properties of samples. A method to separate puree into insoluble particles and serum has also been assessed, and early indications are that the particle weight and volume fraction are related to the rheology of the puree. This hypothesis (hypothesis (ii) stated in section 2.6) will be investigated further in chapter 6 for tomatoes at different stages of ripeness, and in chapter 7 for tomatoes at the same stage of ripeness, with differences in their genes.

The hot break step of the preparation of the puree, whereby the frozen pericarp tissue was heated to 95°C in the microwave prior to blending, produced a more viscous puree than without this heating. In addition to this, the viscosity value was more repeatable between independent puree preparations, shown by a smaller standard deviation for 3 replicate purees. The effect of storing the puree at 4°C was analysed, when the stirred viscosity of red ripe puree was measured at intervals over a one week period. It showed that some viscosity was lost within the first 24 hours after preparation, and then the viscosity remained relatively stable for the next 6 days. This was investigated further by observing the effect of prolonged stirring with the RVA red ripe laboratory prepared hot break puree. The red ripe

puree showed a pronounced loss of viscosity with stirring. Measurement of the particle size before and after stirring in the RVA showed that the larger particles in the red ripe puree were broken into smaller pieces.

The differences between the viscosity values of the tomatoes of different maturity will be investigated and discussed in the next chapter with reference to the quality and quantity of the solid components in the puree.

CHAPTER 6: The effect of ripeness on rheology

6 The effect of ripeness on rheology

6.1 INTRODUCTION

Tomato puree is a suspension of particles within a serum prepared by breaking down of the fruit tissue during a pureeing process. The effect of tomato ripeness on the material properties of the particles and the soluble material, and how these consequently affect the rheology of the puree is not fully understood. This is what this chapter will look at, having developed the methodology for preparing and measuring fundamental rheology and empirical viscosity measurements of laboratory prepared puree in the previous chapter. Therefore this chapter will address aspects of aims (i), (iii) and (iv) stated in section 2.6. As it will soon become clear to the reader of this report, the origin of the observed viscosity of the puree is not immediately obvious, and various factors are considered before conclusions are made as to what the factors are that most contribute to viscosity. This led to the development of hypothesis (ii) section 2.6, which later work tested.

6.2 RESULTS AND DISCUSSION

6.2.1 Effect of puree bulk composition and texture of the fruit on stirred viscosity at different stages of ripeness

The purees were prepared from tomato pericarp tissue at four stages of ripeness, mature green, breaker, pink and red ripe using a hot break process (see methods section 3.2.6.1 for puree preparation method) and a range of parameters measured.

The measurements were total solids, Brix and pH and these were measured for the puree. Aliquots of the puree samples were centrifuged to separate the particle fraction (pellet) from the serum, and the same measurements (total solids, Brix and pH) made on these two parts of the puree, Table 6-1. The table shows that the stirred viscosity of the puree, decreased on ripening. This viscosity measurements were obtained using the RVA (see methods section 3.2.8.4) and the mature green puree had the highest stirred viscosity of over 660cP which decreased to 191cP at the red ripe stage. This confirms the findings of other researchers (Powell et al. 2003, Errington et al. 1998) that have also observed higher viscosity of unripe fruit. Figure 6-1 shows that as the fruits ripened, both the texture of the pericarp and stirred viscosity of the puree decreased.

By comparing data from this current study and previous ones, it is possible to identify what is, and is not likely to have caused the differences in viscosity and texture with ripeness in terms of total solids, soluble and insoluble materials.

Table 6-1 Attributes of the tomato samples after pureeing with (standard deviations) shown

	Stirred viscosity cP	Total solids % (standard deviation)			Brix %			pH		
	Puree	Puree	Pellet	Serum	Puree	Pellet	Serum	Puree	Pellet	Serum
Mature green	663 (42)	5.4 (0.09)	8.7 (0.22)	3.8 (0.01)	4.3	5.2	4.4	4.6	4.5	4.5
Breaker	475 (66)	5.7 (0.01)	9.4 (0.05)	4.4 (0.01)	5.0	5.9	5.2	4.3	4.2	4.2
Pink	254 (41)	5.0 (0.06)	8.9 (0.01)	3.9 (0.01)	4.5	6.2	4.6	4.3	4.2	4.2
Red ripe	191 (22)	5.7 (0.05)	7.3 (0.03)	4.6 (0.01)	5.5	6.4	5.4	4.3	4.3	4.3

*values for pellet and serum are discussed later in this chapter.

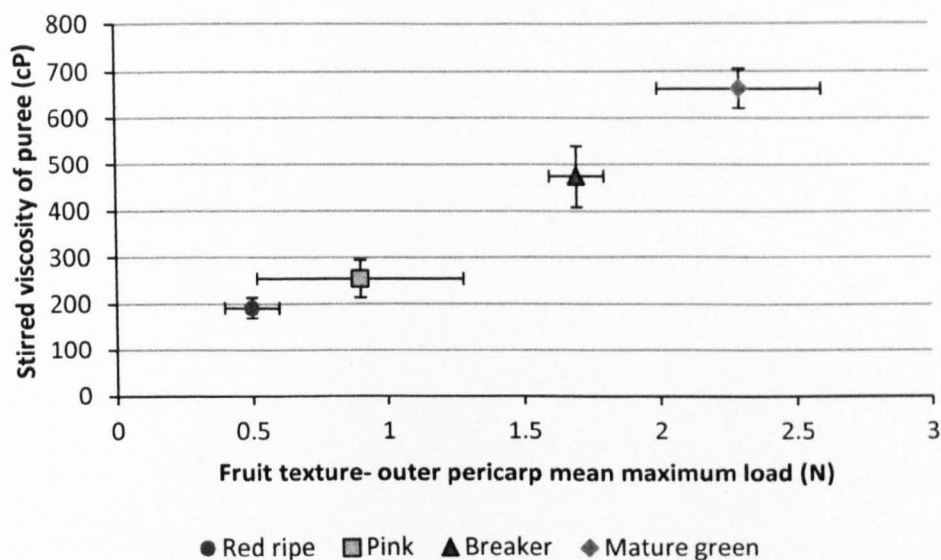


Figure 6-1 Effect of pericarp firmness on the stirred viscosity of puree of tomatoes at four stages of ripeness. Outer pericarp mean maximum load data shown in Table 4-1 and the stirred viscosity values from Table 6-1)

Similarities as well as differences were found when the total solids, simple sugars (soluble solids) and pH measured in the current study was compared with results observed by Winsor et al (1962) in a study of tomato fruit at similar stages of ripeness. These previous results are shown in Table 6-2 so they can be compared with results from the current work. In Table 6-2 it shows the total solids content, reducing sugars and pH (titratable acidity) alter as the fruit ripens (Winsor et al. 1962) and these findings are largely consistent with results reported in Kader et al (1977). In the current study ,however, the total solids and Brix of the puree did not correlate to ripeness, stirred viscosity of the puree or firmness of the fruit shown, indicating that they were not the main contributors to viscosity or fruit firmness. In the current study, the pH decreased from 4.4 in the mature green to 4.0 in the red ripe puree. This is in contrast to the results reported by Winsor et al (1962), where titratable acidity (related to pH) was measured rather than pH. Winsor et al (1962) reported that the titratable acidity decreased from the green-yellow stage to red

stage and in another early study by Kader et al (1977) (data not shown), it was found there was a significant difference between the pH, with values reported of mature green (pH 4.48) and red ripe tomatoes (4.62).

Similar values for total solids were observed in previous studies by (Winsor et al. 1962), but in the previous study total solids and reducing sugars content increase with ripeness. These differences in trend from the current study could be due to the fact that the measurements taken in the current study were of the pericarp tissue, whereas in the previous studies, measurements were taken of the whole fruit, which included skin and locular tissue as well as the pericarp tissues.

Table 6-2 Comparison of total solids, reducing sugars and titratable acidity of purees from whole fruit at five stages of ripeness as reported by (Winsor et al. 1962)

Stage of ripeness	Green	Green-yellow*	Yellow-orange	Orange-red	Red
Total solids g/100ml	4.08	4.51	4.60	4.65	4.70
Reducing sugars g/100ml	2.69	3.07	3.16	3.23	3.27
Titratable acidity (mequiv/100ml)	7.91	8.60	8.21	7.78	7.73

*The green-yellow stage term was assumed to be at approximately the same ripeness as breaker stage

6.2.2 Effect of total solids on tomato rheology

In terms of stirred viscosity, it is unclear how the change in moisture levels (and corresponding total solids) observed with ripeness impacts on the rheology of the system; red ripe tomato puree had the lowest viscosity (at the natural solids content, see Table 6-1), yet one of the highest total solids.

To look further at the relationship between viscosity and solids content purees from breaker and red ripe tomatoes were diluted with water to a range of total solid levels and stirred viscosity measured (Figure 6-2). The results indicate that at the same total solids, the viscosities were greater for the breaker tomato than the red ripe. Ripeness was therefore shown to affect viscosity, and the solids level had some influence on viscosity, but changes in solid levels alone could not explain the differences in viscosity. This could be a sign that the molecular assemblies of hydrocolloids within the total solids may be different during the ripening stage, and had an effect even after the tomato tissue was macerated.

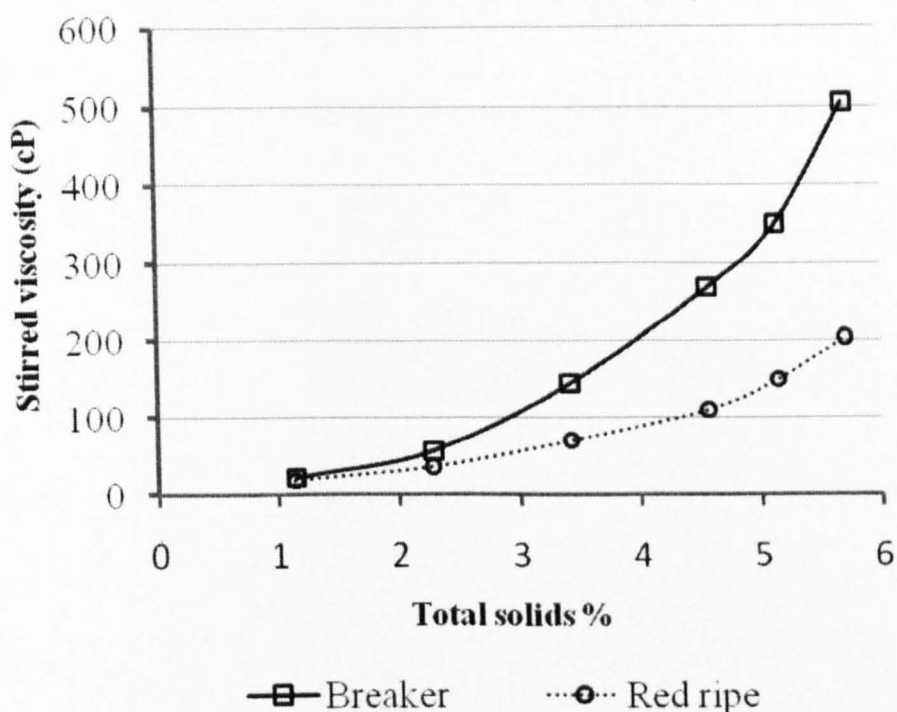


Figure 6-2 Effect of total solids and stage of ripeness on stirred viscosity of tomato puree, diluted with water

Puree from the breaker and red ripe fruits were diluted to the same total solids (5%) and also measured using a serrated parallel plate geometry as described in method section 3.2.8. The suspended solids (pellet) were also measured separately from the puree, by centrifuging the puree and discarding the serum. The extent of the LVR was established for puree and respective pelleted material with a strain sweep at 10 rad/s, Figure 6-3 (see section 2.5.2.2 for explanation of LVR). Puree samples at both stages of ripeness showed the viscoelastic behaviour typical of suspensions, and the linear viscoelastic domain extended to approximately 1% strain. G' (storage modulus) values were larger than G'' (loss modulus) for all samples, which was an indication of the elastic nature of the samples. Pellet data for both the breaker and red ripe are higher than their respective puree data, this was expected due to the more densely packed particles caused by the centrifugation process. A strain of

0.05% was selected for the frequency sweep, Figure 6-4. The findings are similar to those reported for the commercial puree discussed in section 5.2.1

Figure 6-5 shows that the puree and pellet samples were shear thinning. Between the shear rates applied of 1 to 100 s⁻¹, the viscosity of the red ripe puree at 5% total solids was lower than the breaker. This could be an indication of the same trend as described by Yoo and Rao (1994), whereby the smaller particle size of the red ripe (data in Figure 6-13) contributed to the lower viscosity. An interesting result was observed with the pellet viscosity in this large deformation shear; the red ripe pellet showed slightly higher viscosity than the breaker. This is contrary to the small deformation shear results. The higher viscosity of the red ripe pellet than the breaker was unlikely to be due to the total solids of the pellet, which were measured by oven drying. The complex nature of the puree is demonstrated by these findings, warranting further investigation of the liquid and solid phases of the puree and the soluble and insoluble solid fractions that make up the total solids.

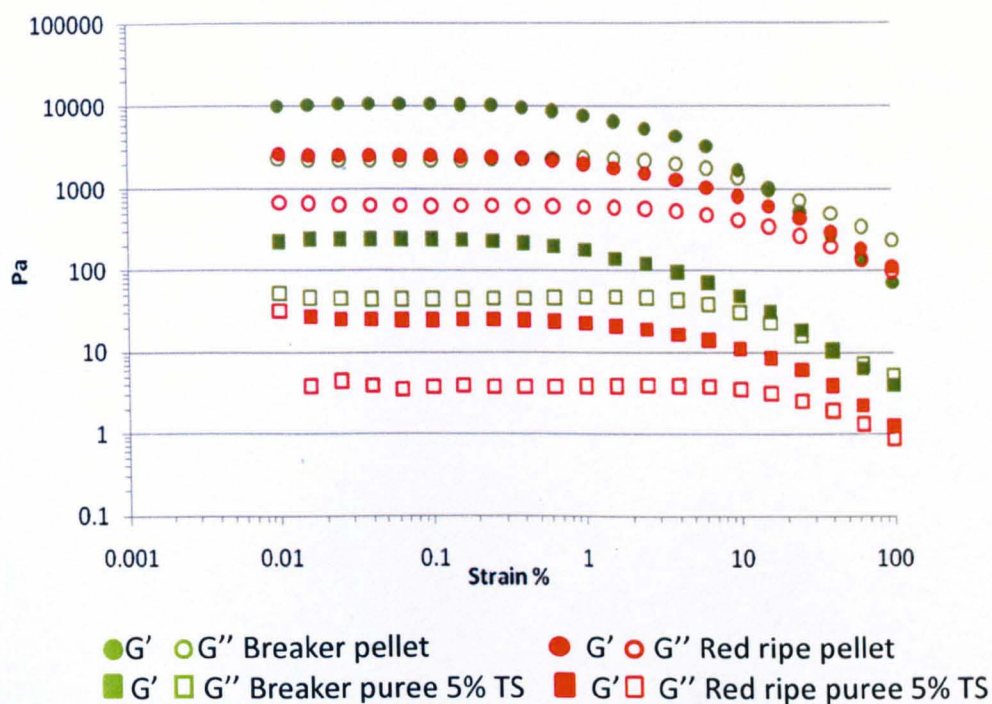


Figure 6-3 Strain (amplitude) sweep performed at 10 rad/s for puree (5% TS) and the centrifuged pellet of breaker and red ripe puree. Elastic/storage modulus G' (filled symbols), and the loss modulus G'' (open symbols)

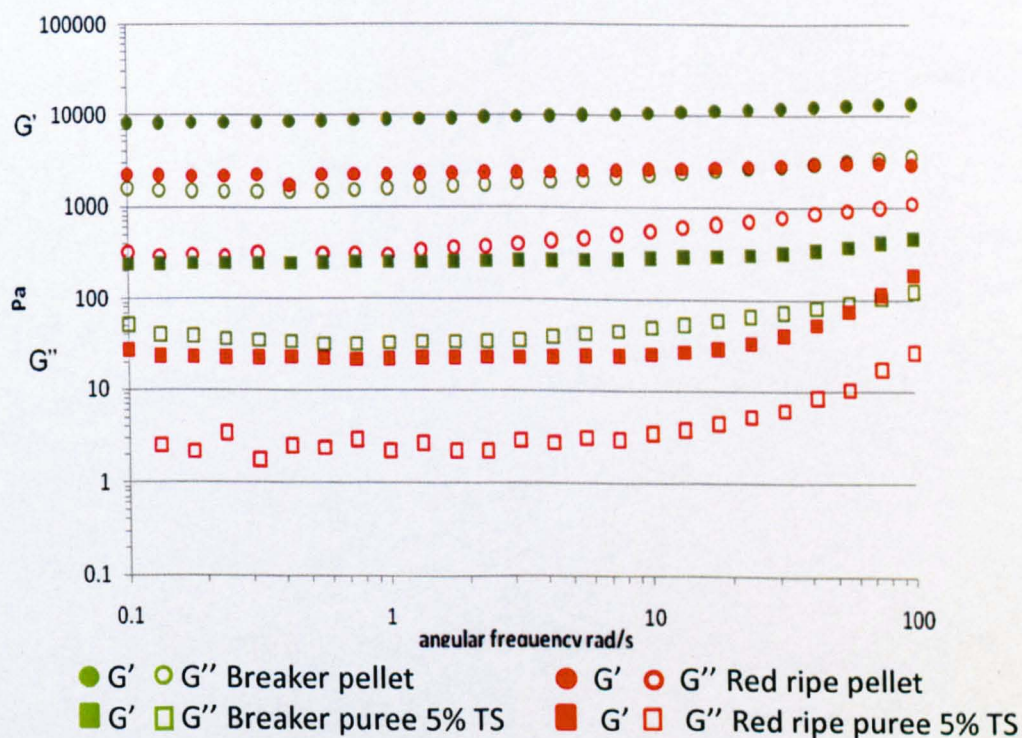


Figure 6-4 Frequency sweep at 0.05% Strain for puree (5% TS) and the centrifuged pellet of breaker and red ripe puree with G' represented by filled symbols, G'' with open symbols

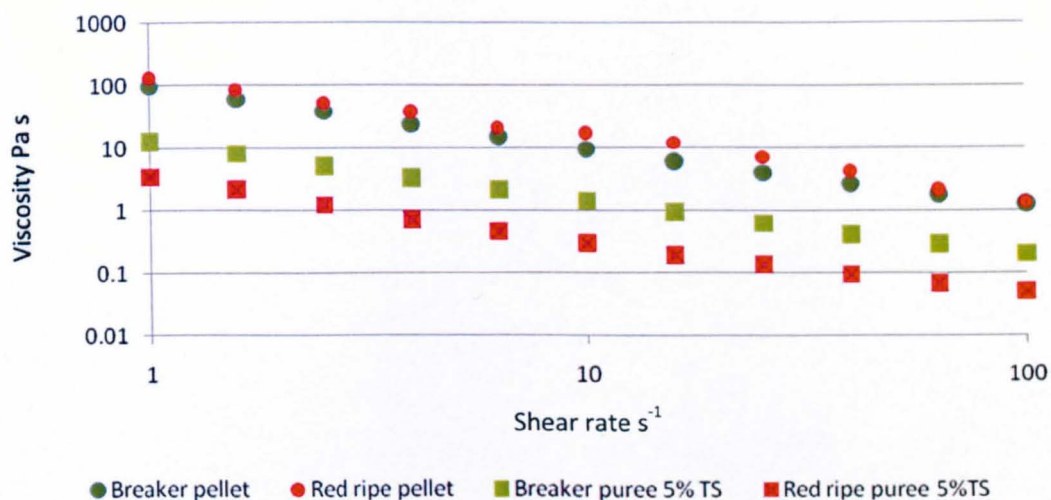


Figure 6-5 Flow curve 1-100 s^{-1} for puree (5% TS) and the centrifuged pellet of breaker and red ripe puree

6.2.3 The effect of soluble solids on tomato rheology

Because tomato sera contain soluble pectins as well as simple sugars and acid, (Gancedo and Luh, 1986) it was pertinent to determine the contribution of the serum viscosity to the total puree viscosity. The undiluted puree was centrifuged to separate the serum from the pellet and viscosity measured. This was initially measured at 10 s^{-1} using the double gap geometry with the MCR rheometer, displayed as a function of serum soluble solids, (Brix), Figure 6-6. At this shear rate, the viscosities of the sera were low, and similar to sucrose solution at the same concentration, but did not follow the expected trend of mature green being the highest and red ripe the lowest, in line with the breakdown of pectin molecules on ripening (as reviewed by Brummell and Harpster (2001)). It is likely that the higher soluble sugar content (Brix) of the more ripened fruit caused some of the increase in total solids of the serum on ripeness, and the majority of the viscosity.

The soluble solids of the tomato serum, expressed as Brix was higher than the total solids, measured by oven drying. This was perhaps an indication that some of the smaller molecules are heat labile and therefore are lost during the oven drying process at 105°C.

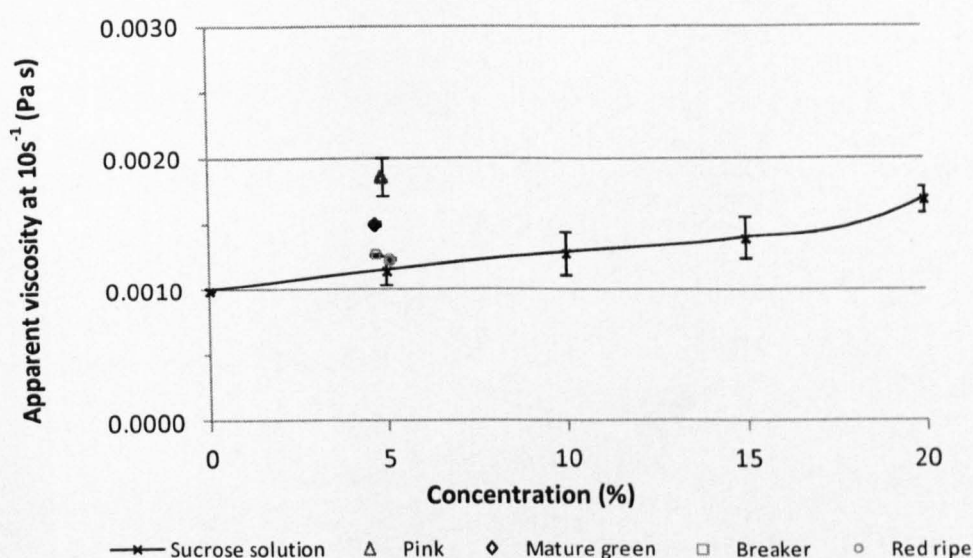


Figure 6-6 Apparent viscosity of tomato serum as a function of the Brix (%) and apparent viscosity of sucrose solutions as a function of the sucrose concentration (% w/w)

The contribution of the serum on tomato puree viscosity was investigated when combined with the pellet fraction. In order to prepare the samples, the pellet fraction from the red ripe puree was first separated from the serum by centrifugation and was then resuspended at a ratio of 32:68 % by weight pellet: liquid (serum) phase, with either 4.4% w/w sucrose solution, water or the original serum. The concentration of the sucrose solution was chosen to match the solids content of the serum and the proportion of pellet to serum was comparable to the amounts naturally occurring in the tomato puree. The stirred viscosity of these

three samples was measured (Table 6-3). The fact that stirred viscosity is independent of whether the suspension medium constituted sucrose solution or serum phase again showed that little contribution to the viscosity of the puree is coming from the tomato sera as shown previously by Tanglertpaibul and Rao (1987), and that the soluble pectin in the serum had minimal contribution to viscosity. The viscosity was lower in the samples resuspended in water, a fluid of lower osmotic strength than the other two suspending fluids, indicates that the osmotic strength of the suspending medium may have an influence. It is hypothesised that the lower viscosity is due to contraction of the particles.

Table 6-3 Effect of liquid phase (68% by weight) on stirred viscosity, and standard deviation of pellet (32% by weight) obtained from red ripe tomato puree

Liquid phase	Total solids of liquid phase%	Stirred viscosity cP (standard deviation) of 3 replicates*
Serum	4.4	212 (4.8) ^a
Sucrose solution	4.4	211 (10.4) ^a
Water	0.0	193 (2.0) ^b

* Samples with same letter are not significantly different P>0.05

6.2.4 Effect of particles (pelleted material) on tomato rheology

The relative amounts of particles and serum, after centrifuging the tomato purees at natural total solids, were calculated as the ratio of pelletable particle material, compared to the weight and volume of the total sample, Table 6-4. In Figure 6-7 a photograph is shown of the pelleted material after centrifugation of 50g of tomato puree with the sera removed, for a visual guide to the quantity of pelleted material obtained at each ripeness stage. The trend for the particle to sample volume ratio

does not directly reflect the viscosity decrease with ripening of the fruit. If the volume, or weight of pellet was the most significant factor in tomato puree viscosity, it would follow that the amount of pellet obtained from the purees of tomato at the different states of ripeness would be; mature green>breaker>pink>red ripe, whereas clearly this is not the case. A reason for this variation could be that the sedimentation of particles is dependent on several factors such as liquid viscosity, particle size and density, fraction of dissolved solids and gravitational force applied to hydrated particles, and these factors may have varied with ripeness. However, it was reported in Errington et al, (1998) that viscosity values and particle volume of puree made from genetically modified tomatoes with suppressed ripening related pectic enzymes did not correlate entirely. They concluded that as well as the amount of solid pelletable material, the structure of the solid material could be significant.

Table 6-4 The particle to total sample weight ratio and the particle to total sample volume ratio of tomato purees (see section 3.2.11.2 for method details)

Ratio	Mature green	Breaker	Pink	Red ripe
Particle:total sample weight	0.26 (0.03)	0.23 (0.01)	0.17 (0.01)	0.28 (0.01)
Particle:total sample volume	0.28 (0.03)	0.24 (0.01)	0.18 (0.01)	0.30 (0.01)

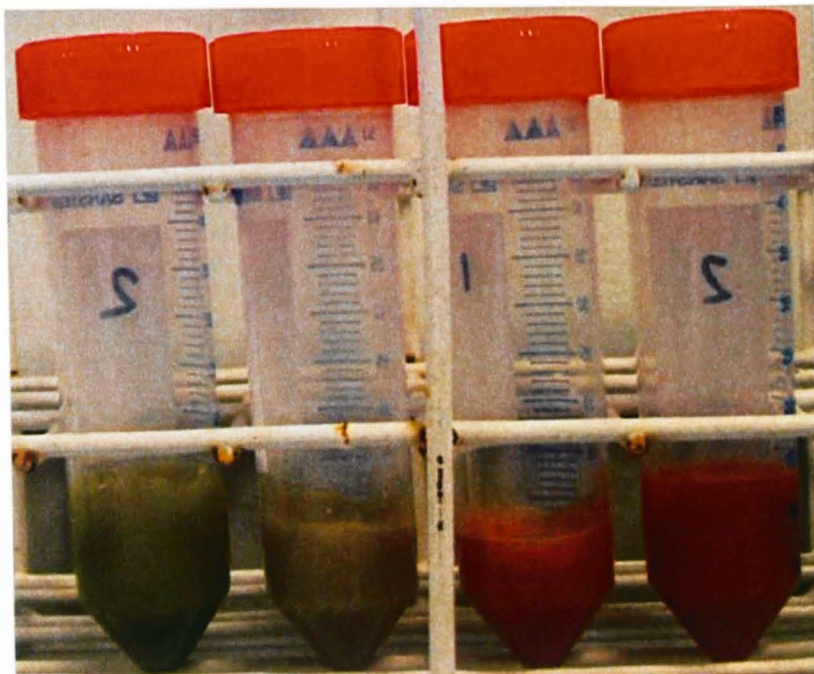


Figure 6-7 Pelleted material in 50ml tubes after centrifugation and removal of serum (mature green, breaker, pink and red ripe, left to right).

6.2.4.1 *Effect of pellet concentration and interaction of particles and soluble material on tomato rheology*

To gain understanding of the effect of particle (pellet) concentration and interaction between particles and soluble solids, the pellet from the red ripe tomato was diluted with sucrose solutions of varying concentrations. The concentration of sucrose and amount of pellet were adjusted to maintain the overall total solids of the samples between 3.9 and 7.9%, with the pellet contributing to 16 to 48% by weight of the sample. Stirred viscosity of the resulting samples was measured (Figure 6-8). For lower sucrose levels contributing to the total solids content, and increasing particle concentration (> 24% and most noticeable at the highest pellet content of 48%), suspension viscosity was affected by the sucrose content of the serum. However, with all sucrose concentrations measured, the amount of pellet was the main source of the viscosity. Similar results were observed by

Tanglertpaibul and Rao (1987) in that pellet (pulp) to liquid ratio is strongly related to apparent viscosity of tomato puree (that is, if progressively more particle fraction is added to a set amount of serum, the viscosity will increase).

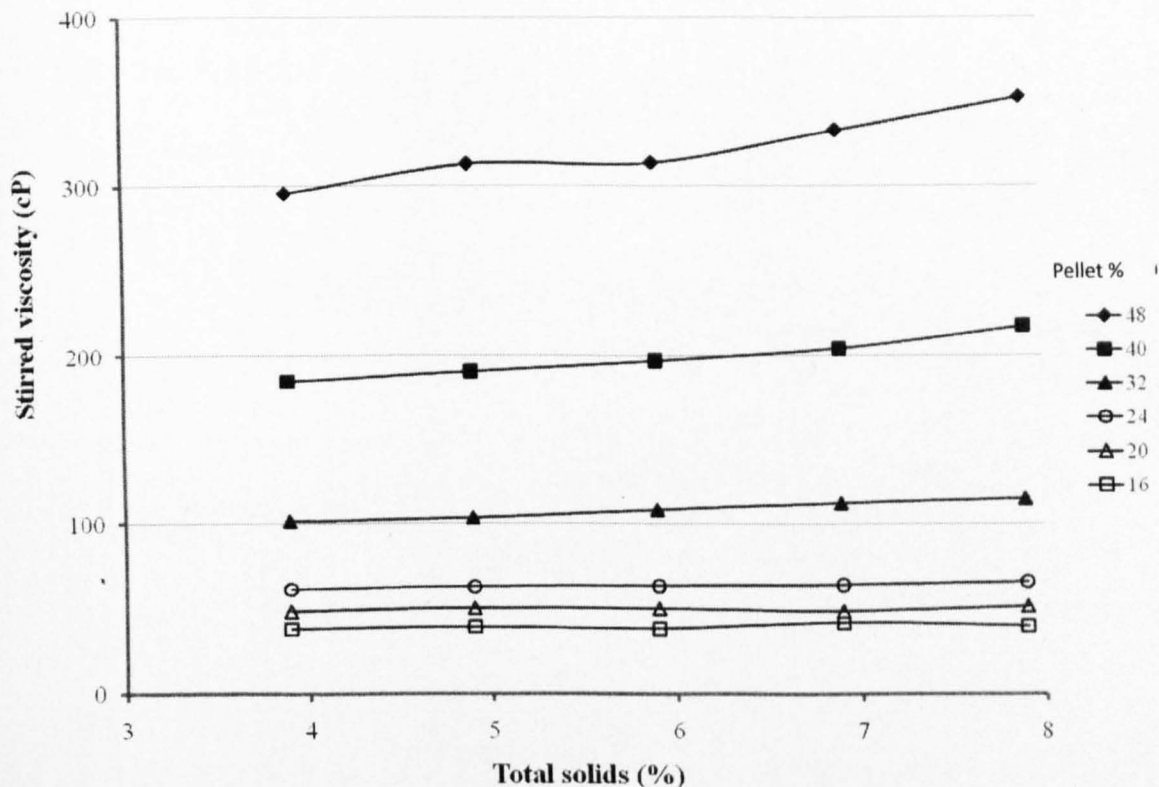


Figure 6-8 Effect of percentage of pellet (16-48%) obtained by centrifugation of red ripe puree, sucrose concentration and total solids on stirred viscosity.

The pellets of the other tomato samples (mature green, breaker and pink) were also diluted with sucrose solutions of a range of concentrations, at the same ratios of pellet to liquid by weight as the red ripe (16, 20, 24, 32, 40, 48 % by pellet weight). The stirred viscosity for all four stages of ripeness were plotted as a function of pellet total solids Figure 6-9, but this time the error bars were representative of the standard deviations between measurements of samples with the same total solids, but different sucrose concentrations in the liquid phase. This was done because the sucrose concentration only had a small effect on viscosity over the range of pellet

concentrations. Within the range of pellet concentrations measured, the results show that the more ripened the fruit, the lower the viscosity, although there is only a slight difference between the breaker and the pink samples. The increase in viscosity with pellet total solids was non-linear due to the inter-particle interactions more likely at higher particle concentrations. The effect of particle:particle interactions has been discussed in the literature review with reference to the c^* concentration, section 2.5.2.

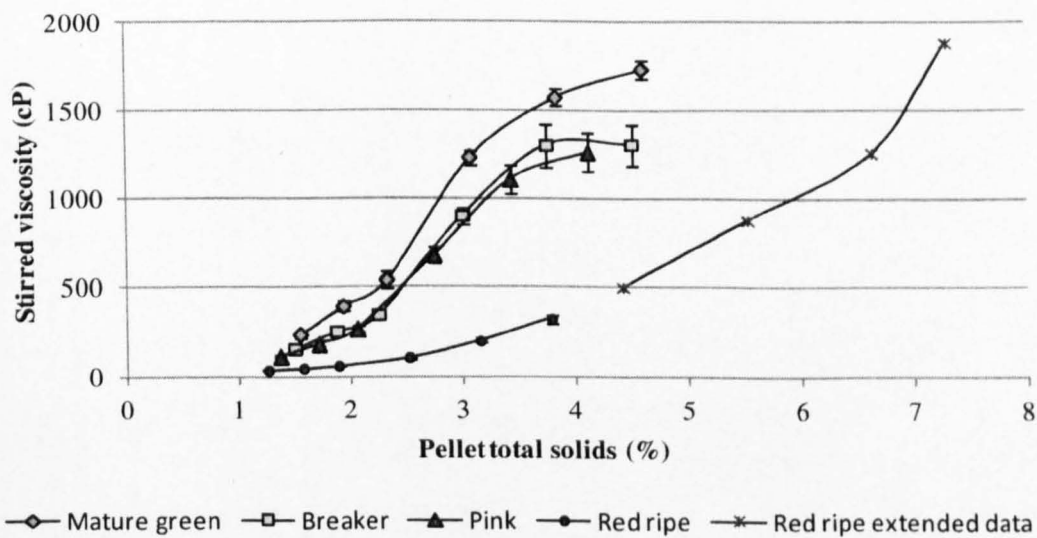


Figure 6-9 Effect of pellet (total solids) on stirred viscosity of pelleted material diluted with sucrose solutions of varying concentrations

6.2.4.2 Effect of pellet composition on tomato rheology

It is clear from Figure 6-9 that increasing the amount of pellet (particle material) increases viscosity, what is still not clear is why maturity of the fruit also has an effect. The total solids of the pelletable material is shown in Table 6-1, and varied from 7.3-8.9 percent, with no clear correlation between ripeness and pellet total

solids. The next step was to investigate the effect of the bulk composition of the solids in the pellet.

As such, using the values quoted in Table 6-1 and Table 6-4 the amounts of the different components were calculated as a percentage of the total puree. This data are shown in Table 6-5.

Table 6-5 Calculated percentage of water, soluble and insoluble solids in the particle (pelletable) and serum phases of tomato puree at four stages of fruit ripeness, assuming the pellet weight fractions in *Table 6-4* and the total solids and brix contents in the pellet and serum from *Table 6-1*.

Component	Water		Insoluble material (total solids - Brix)		Soluble material	
	Particle fraction%	Serum fraction%	Particle fraction%	Serum fraction%	Particle fraction%	Serum fraction%
Mature green	23.7	71.2	0.9	-	1.4	3.3
Breaker	20.8	73.6	0.8	-	1.4	4.0
Pink	15.5	79.8	0.5	-	1.1	3.8
Red ripe	26.0	68.7	0.3	-	1.8	3.9

[Explanation of how the values were calculated. For mature green sample, in 100g of puree, the pellet (particle) weight is assumed to be 26g of the total weight (0.26 ratio, *Table 6-4*). The mature green pellet total solids is 8.7% (see *Table 6-1*). Therefore the total solids in the pellet is 2.3% of the total weight, and thus it is calculated that water in the 26g of particle fraction 23.7g. The % Brix in the pellet was 5.2% (*Table 6-1*). 5.2% of 26g is 1.4%. 2.3-1.4=0.9% insoluble solids. All solids in the serum assumed to be soluble.]

Using the stirred viscosity values from Figure 6-9, viscosity was plotted as a function of pellet insoluble solids, calculated by subtracting the pellet Brix from the pellet total solids, Figure 6-10. Although the results do not superimpose exactly, there

appears to be a correlation between pellet insoluble solids and stirred viscosity for the mature green, breaker and pink tomato puree, thereby suggesting that the amount of insoluble solids was an important factor in tomato puree viscosity. The theory for this could be the quality and quantity of the cell wall structures that change during ripening affect viscosity for these three samples, in terms of hemicelluloses and pectins being of high or low molecular weight, as well as the amount of insoluble solids was important. When the stirred viscosity is plotted as a function of the pellet Brix, Figure 6-11, differences between the four stages of ripeness is again observed.

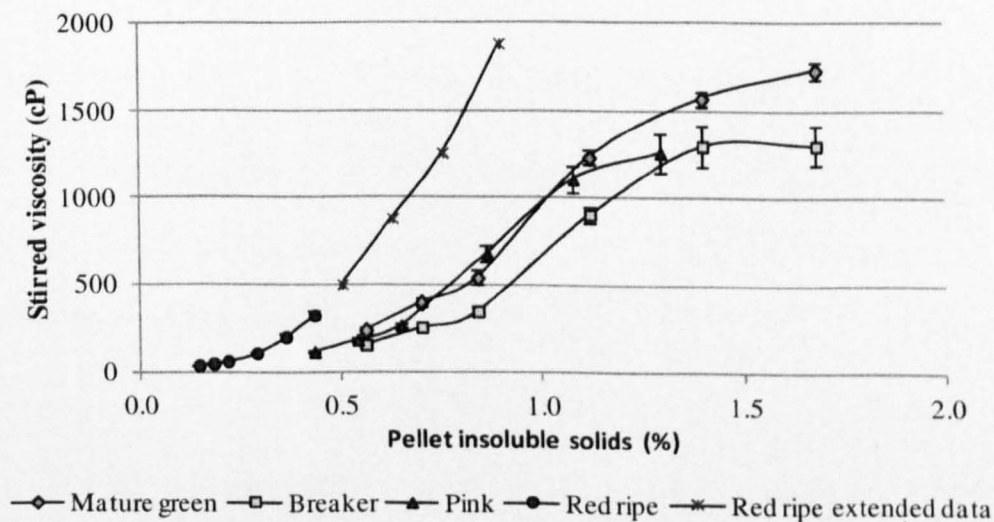


Figure 6-10 Effect of pellet (insoluble solids) on stirred viscosity of pelleted material diluted with sucrose solutions of varying concentrations

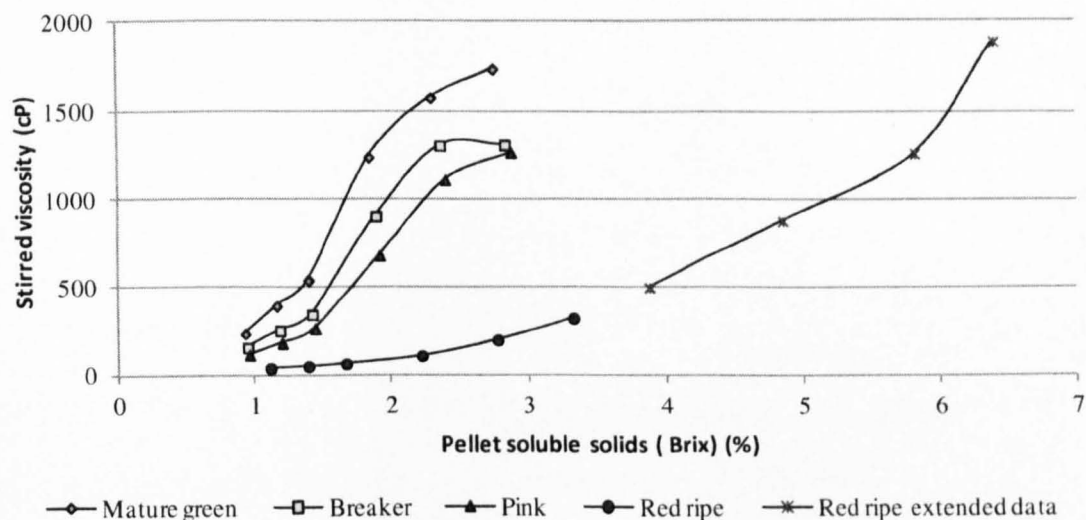


Figure 6-11 Effect of pellet (soluble solids, Brix) on stirred viscosity of pelleted material diluted with sucrose solutions of varying concentrations

6.2.5 Particle nature of the pellet

The pellet fraction containing the particles was obviously key to the viscosity of the puree. As viewed under a light microscope (Figure 6-12), it was observed that the particles are mainly single and fragmented cells, with the mean diameter of a single tomato cell at approximately 400 μm in diameter. Quantitative data for size distribution measured by laser light scattering (see method section 3.2.9) (Figure 6-13) show that as the tomato ripened from green to red, particles were smaller on average and of a broader size distribution. In the red ripe sample more of the particles were broken cell fragments, perhaps due to the disassembly of polysaccharide structures in the cell wall (see section 2.3.4) making them more susceptible to breakdown during pureeing. The size distribution for the mature green puree extends to sizes much larger than the diameter of a single cell ($\sim 400 \mu\text{m}$) indicating that some clusters of cells remained in the puree due to strong cell to cell adhesion of the middle lamella pectins. An example of cluster of green tomato cells and fragmented red ripe cells in pureed samples is shown in Figure

6-14. The mean diameters for the particles in the 4 puree samples were 893 μ m (mature green), 542 μ m (breaker), 377 μ m (pink), and 262 μ m (red ripe).

In a previous study using plant based suspensions similar to tomato puree (Sato and Cunha, 2009), a large particle size distribution has been associated with a lower viscosity, whereas larger particles caused increased viscosity. The results shown in this chapter could indicate a similar trend.

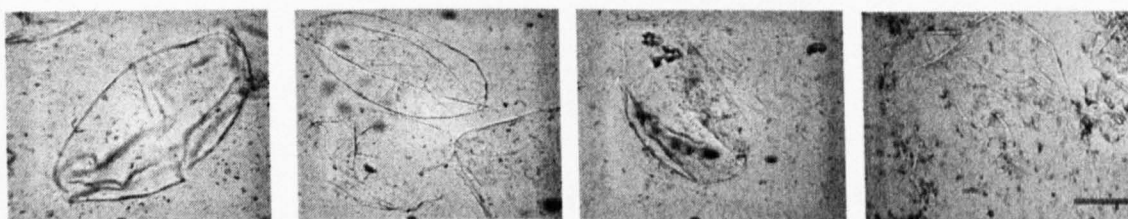


Figure 6-12 Light microscope images of mature green, breaker, pink and red ripe tomato puree (left to right). Scale bar 100 μ m.

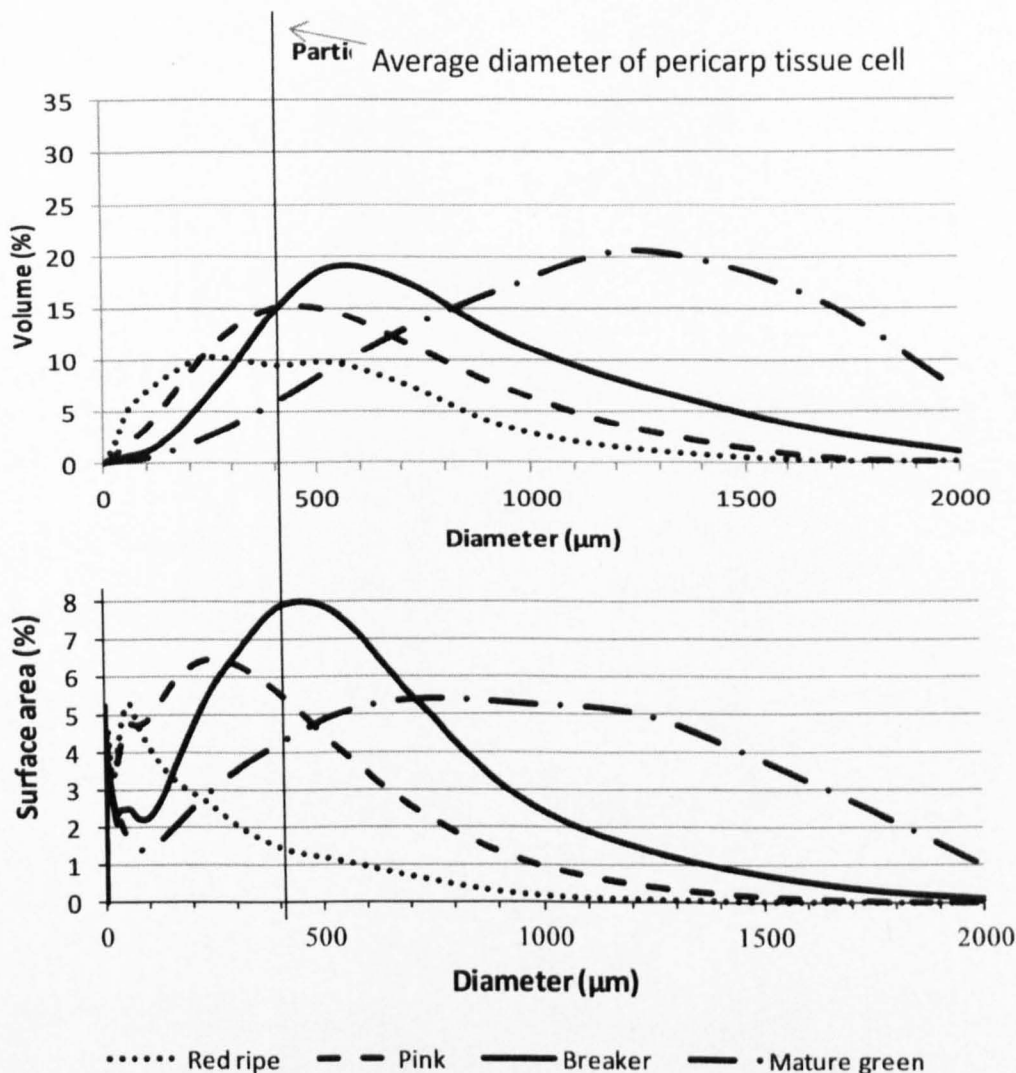


Figure 6-13 Particle size distributions of puree samples from mature green, breaker, pink and red ripe tomatoes as determined by laser light scattering displayed as volume (top) and surface area (bottom). The estimated diameter of a typical intact cell is indicated on both charts.

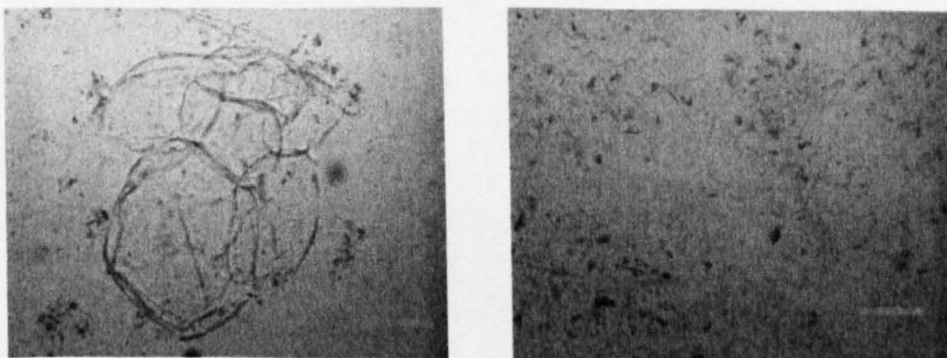


Figure 6-14 Light microscopy images of a particle consisting of a cluster of cells particles in mature green puree (left) compared with the fragments of cells more often observed in the red ripe (right) tomato puree. Scale bar 100μm.

6.2.6 Macromolecular construction of the particle material

The quality of the middle lamella pectin, the 'cement' between adjoining cells was assessed rheologically. This was done to give an initial indication of its structural strength when extracted from tomatoes at different stages of ripeness. The pectin was extracted from the isolated cell wall material (AIS) from mature green and red ripe tomatoes using water, then the chelating agent, CDTA

(cyclohexanediaminetetraacetic acid), as described in the methods section 3.2.7.

The results show in Table 6-6 that not only did mature green fruits have more cell wall material extracted using acetone and ethanol, but also that the percentage of water and CDTA soluble pectins was different.

The viscoelastic properties of the CDTA soluble pectin extract were measured using a small angled cone and plate geometry at 0.5 % strain, at a concentration of 4% w/w, Figure 6-15. These were initial results for rheology, and the parameters would need refining for improved data however, it can be seen that the pectin extracted from the mature green tomato has a higher overall modulus than the red ripe at the same concentration, with the G' predominating for most of the frequencies tested for the green, indicating a more gel like structure. This pectin rich layer has been shown to decrease in molecular weight on ripening (as reviewed by Brummell and Harpster (2001), see section 2.3.4 of the literature review) and the strength appears to be compromised in the more ripened fruits, meaning that during the processing, the cells are more easily separated, thus more cell clusters remained in the mature green puree than the red.

Observations were made of the pericarp tissue after they had been heated in the microwave to 95°C but prior to pureeing and images are shown in Figure 6-16. It can be seen that after the heating process, the mature green pericarp tissue was relatively intact, whereas the pink and red ripe fruits had disintegrated with heating, suggesting that the cell:cell bonding was progressively weaker in the more ripened fruit.

Table 6-6 Cell wall material (AIS), water and CDTA soluble material in mature green and red ripe tomato pericarp

	Cell wall material (AIS) as a percentage of the pericarp	Water soluble material % (of the cell wall material)	CDTA-soluble material % (of the cell wall material)
Mature green	1.49	6.1	28.9
Red ripe	0.67	8.7	27.5

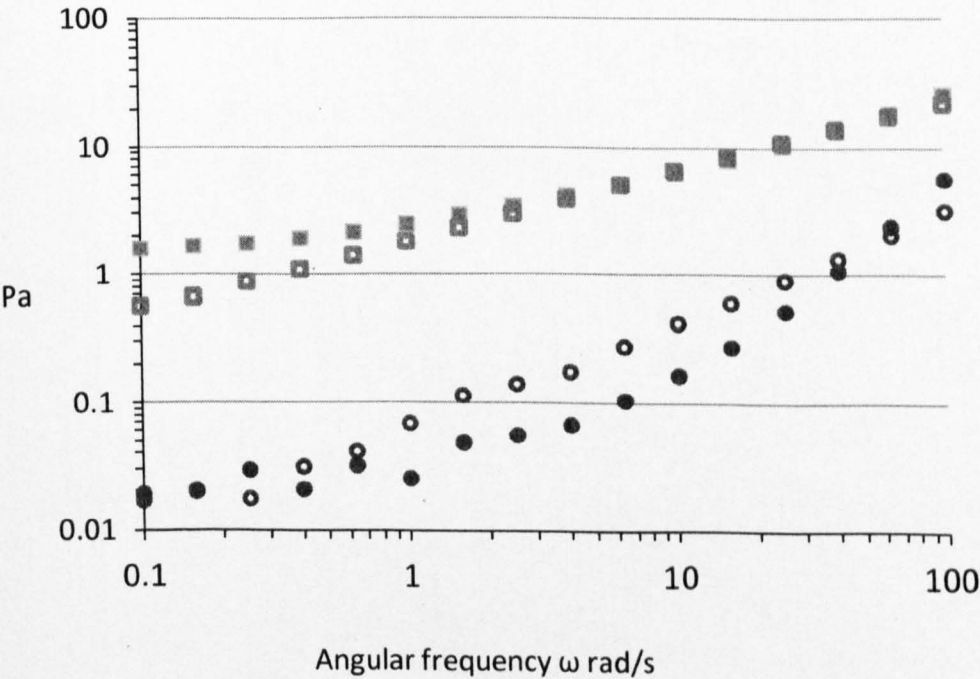


Figure 6-15 Rheology of 4% pectin extracted with CDTA (assumed to be from the middle lamella) of mature green (squares) and red ripe (circles) tomatoes. G' closed symbols, G'' open symbols.



Figure 6-16 Mature green, pink and red ripe tomato pericarp (left to right) after microwaving to 95°C.

[The tomatoes were microwaved from frozen. The mature green tissue is relatively intact after this hot break process, and as the fruit ripened showed tissue breakdown. Fruit were blended after this heat treatment to make the puree.]

6.2.7 Flexibility of the particles

The viscosity will be affected by the mechanical properties of the particles especially as the volume fraction of the dispersed phase increases towards the possible maximum. This was assessed by preparing suspensions of increasing particle volume fraction and determining relative viscosity at 10 s^{-1} (serrated parallel plate geometry), Figure 6-17. Sucrose solutions of approximately 5% w/w were used as the continuous phase. It should be noted that the viscosity data were to some extent influenced by the choice of gap height in the parallel plate geometry. However, the effect was not of several orders of magnitude, thereby did not invalidate the finding that, the puree particles are deformable and behave quite differently from what would be expected for a solid sphere suspension. The relative viscosity behaviour for a suspension can be modelled with the Krieger Dougherty equation (Krieger and Dougherty, 1959) (see insert in Figure 6-17), and the behaviour for solid spheres assuming a maximum packing volume of $\phi_m = 0.62$ and for which the intrinsic viscosity = 2.5 has been included with the experimental data for tomato puree based suspensions. The differences are striking and much higher

relative viscosities as well as packing fractions as indicated by the packing fraction ratio ϕ/ϕ_m exceeding values of unity achieved. At low particle concentration pellets from different sources of tomatoes seem to fit within the same range of viscosities, but at higher concentrations the values are very different, this may imply that it is a change in the robustness/ solidity of the particles that is the biggest change through ripening, which reflected the firmness of the flesh before pureeing.

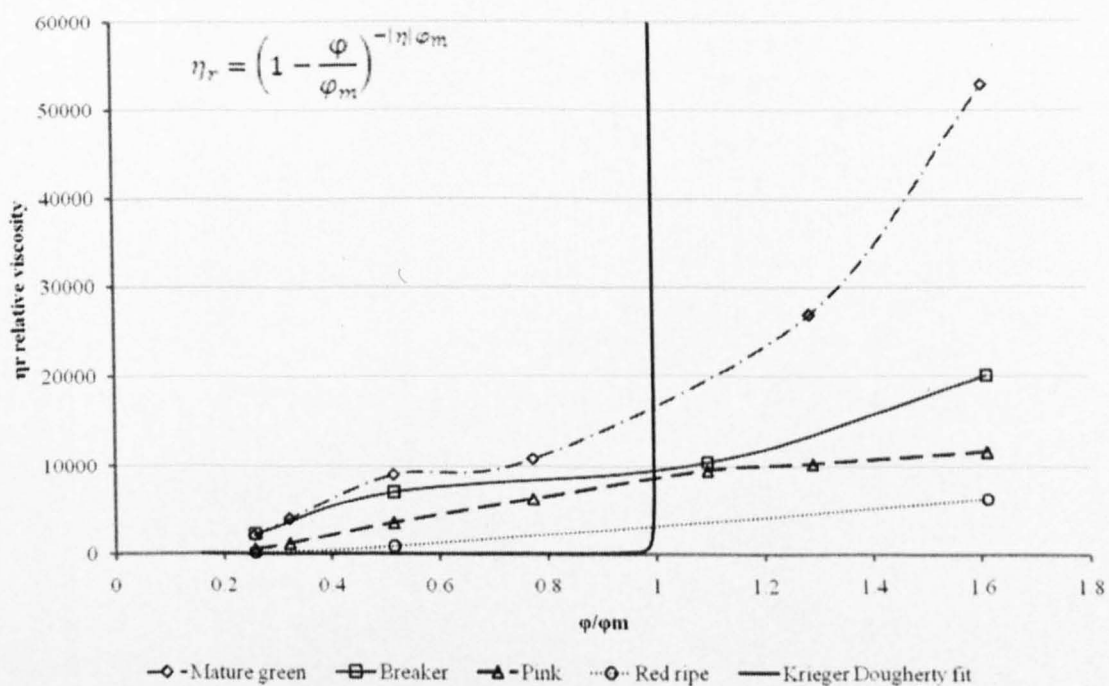


Figure 6-17 Relative viscosity η_r of pelleted material from tomato puree measured using a parallel plate geometry, compared with the Krieger Dougherty fit (see insert in graph for equation) for solid spheres whereby with $\phi_m = 0.62$ and $[\eta] = 2.5$.
 [Note that relative particle phase volume for pelleted material was calculated with $\phi_m = 0.62$. The Krieger Dougherty fit shows relative viscosity increasing at the maximum packing fraction for solid spheres $(1 - (0.62/0.62))$, where ϕ was assumed to be the same as % of pellet by weight of sample.]

6.2.8 Resistance of the particles to damage by shear

Particle size analysis of breaker and red ripe puree before and after shear in the RVA (Figure 6-18) shows that the more ripened fruit particles were more susceptible to damage and break-up on shearing, again indicating the strength of the mechanical properties were reduced on ripening, again suggesting that there is a difference in the robustness or solidity of the particles on ripening.

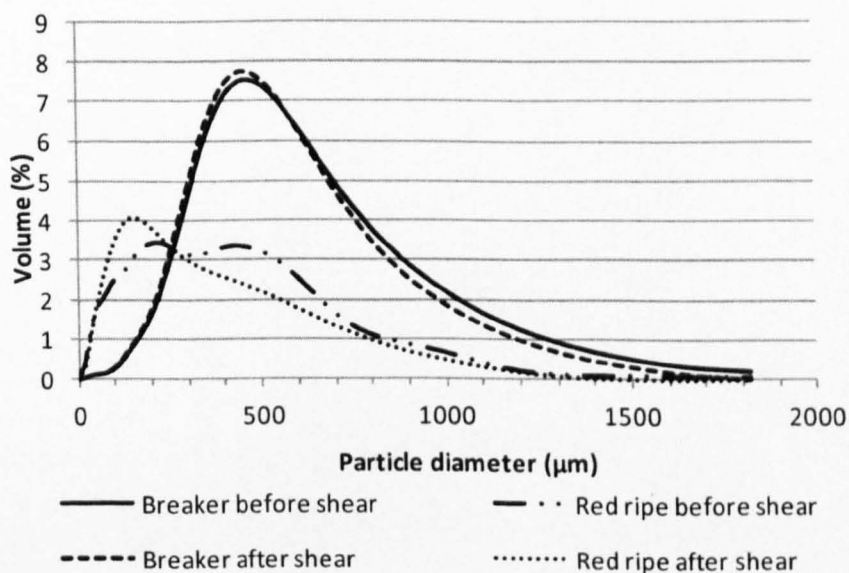


Figure 6-18 Red ripe and breaker particle size before and after shear in the RVA, measured by laser light scattering

6.3 CONCLUSIONS

The contribution of the particles and soluble material to tomato puree viscosity has been studied with reference to the varying composition of solids of tomato fruit at four stages of ripeness; mature green, breaker, pink and red ripe. Initial work in this chapter lead to the hypothesis (see hypothesis (ii), section 2.6) that the viscosity of the tomato puree was dominated by the insoluble suspended solid fraction (i.e. the particles), rather than the total solids. When purees from the red ripe and the

breaker fruit were diluted to a range of total solids content, both samples decreased in viscosity. In these systems the particles and the total solids would be reduced and the hypothesis (hypothesis (ii) section 2.6) would be justified. On further investigation, the viscosity of the puree was shown to be affected only to a small degree by the viscosity of the serum. Again, this indicates the dominance of the particles as suggested in hypothesis (ii)) would be justified. The absence of soluble sugars within the (serum) liquid phase caused a slight decrease in viscosity, possibly due to osmotic potential on the particles (or due to the viscosity of water, the continuous phase being lower than the viscosity of sucrose solution or tomato serum). Most dominant for viscosity was the particle fraction and therefore hypothesis (ii) is confirmed.

The particle fraction was affected by the maturity of the fruit which in turn was associated with changes in texture of the flesh before pureeing and particle size and size distribution of the puree. This supports hypothesis (i) (section 2.6) that factors associated with ripeness, including texture of the tomato pericarp correlate with the viscosity of the pureed pericarp tissue. Addition of more of the particle (pellet) fraction increased viscosity, but the particles do not behave as rigid spheres, as shown by the large departure from the corresponding Krieger Dougherty fit; ripened fruit deviating the furthest. This suggests the more ripened fruit has softer particles, which was shown to have softer tissue before the pureeing process. This may also have made the tissue more susceptible to rupture into smaller particles during pureeing.

Consumers prefer the flavour and colour of ripened tomatoes, but pureed green unripe fruit have been shown to offer more potential as an effective natural viscosifier for foods such as soups and sauces. New varieties of tomato can be developed with the study of fruit from the *S pennellii* introgression library where cultivated tomatoes have been crossed in a highly specific way with a wild type tomato, *S. pennellii* (Eshed and Zamir, 1995). Fruit displaying desirable characteristics (e.g. increased viscosity) when ripe can then be further analysed to determine the responsible gene(s). The next chapter will therefore focus on researching tomato with variations in their genes rather than ripeness.

CHAPTER 7: Introgression line viscosity screen

7 Introgression line viscosity screen

7.1 INTRODUCTION

The previous chapter describes the use of tomatoes at different stages of ripeness to investigate some of the important attributes of tomato viscosity. These results confirmed hypotheses (i) and (ii) (section 2.6) that puree viscosity is dominated by the properties of the particles, and these properties correlate with fruit texture. However, the results so far were obtained on tomatoes at different stages of ripeness. The study required further investigation to see if other factors, besides maturity affected texture and puree viscosity to the same extent. This will challenge hypothesis (iv) (section 2.6) where tomatoes from the *S. pennellii* introgression library at one stage of ripeness were analysed. These samples were genetically different from each other, thereby potentially allowing for control of phenotypes, such as viscosity at the molecular level. The first part of this chapter therefore starts with the development of a viscosity screen of the *S. pennellii* introgression lines.

In this chapter, a viscosity screen was developed and used on a population of *S. pennellii* introgression lines (ILs), at the same stage of ripeness. They were harvested and processed in a laboratory hot break process and viscosity determined, as described in the methods chapter. The tomatoes were grown under controlled conditions in the glasshouses at Sutton Bonington, harvested seven days after the

first flush of colour from green to pink, and were analysed to determine whether gene expression could be related to puree viscosity. The latter part of this chapter shows the results for the viscosity and texture of a firm fleshed fruit selected from the viscosity screen, and compares this to the control M82.

The wider objective of the work was to aid a more direct approach to developing breeds of tomatoes with high viscosity.

The aims of the work reported in this chapter were;

- i) To develop a screen to quickly compare the viscosity of small samples of tomato puree of a large number of samples
- ii) To compare the viscosity of puree made from tomatoes from the *S. pennellii* introgression line collection
- iii) To record and discuss viscosity in comparison with factors known to affect viscosity
- iv) To compare texture and viscosity of the control (M82) with a firm textured fruit (IL 3-4)

7.2 RESULTS AND DISCUSSION

7.2.1 Preliminary results – testing the puree preparation method and analysis

A feature of using the introgression lines was that few samples are available for each line and many lines need to be assessed. Also growth of these samples is labour intensive and the season is short for successful cropping. It was therefore important that some preliminary work took place to show that the puree

preparation and measurement methods produced consistent results. General commercial red ripe tomatoes were therefore purchased from a local supermarket to use in perfecting the testing regimes. A batch of these commercial tomatoes was peeled and the pericarp tissue diced and combined. This bulk sample was then separated into the six subsamples. Each subsample was microwaved and blended according to the protocol given in the methods chapter section 3.2.6.2. The samples were diluted with water to 3.5% solids, thereby assuring that all samples could be tested at the same solids content. The stirred viscosity measured was then measured using the RVA (see method section 3.2.8.4). Table 7-1 shows the mean stirred viscosity for the six replicates of puree.

The viscosity values showed a coefficient of variation of <10%, which was considered acceptable for this type of assessment and shows that microwaving of the different replicates had a reproducible effect on the measured viscosity. It was therefore concluded that this method of sample preparation was acceptable.

Table 7-1 Effect of the puree preparation on total solids and viscosity of red ripe puree made with supermarket tomatoes when diluted to 3.5% total solids (6 replicates)

Initial total solids % with (standard deviation)	Stirred viscosity cP at 3.5% total solids with (standard deviation)
7.6 (0.3)	42 (3.6)

The effect individual fruit on stirred viscosity was tested by preparing six samples of puree from six individual tomato fruits from a different batch from that used in the previous section, Table 7-2. There were variations in the total solids content and as

it is known that concentration may play an important role in viscosity measurements (see Figure 6-2) the decision was made to look at all puree at the same solids level. As the lowest solids content were above 3.5%, this concentration was chosen so that all samples could be diluted to this level. The total solids of each tomato were measured and therefore the samples were diluted individually to achieve a solids content of 3.5%. The viscosity values showed a coefficient of variation of 26% and may reflect differences due to the natural variation between individual tomatoes. Interestingly the mean values for these individual samples show low solid contents, but much higher viscosities compared to the samples used for the bulk assessment (Table 7-1).

The batch of samples used for measures of individual performance was assessed at the red ripe and at the mature green stage. Despite the variation in the viscosities when assessing the individual samples, there is a statistical difference ($p < 1\%$) between the viscosity of the red ripe tomato puree and mature green tomato puree, Table 7-2. This indicates that the method is sufficiently robust to differentiate between samples of different ripeness, when they are compared at matched solids content.

Table 7-2 Effect of individual fruit and puree preparation on mature green and red ripe commercial tomato puree stirred viscosity values when diluted to 3.5% TS *

Tomato type	Initial total solids % with (standard deviation)	Stirred viscosity cP at 3.5% TS with (standard deviation)
Mature green (2 replicates)	4.6 (0.1)	192 (11)
Red ripe (6 replicates)	4.0 (0.3)	110 (29)

* t-test carried out. Significant difference at ($p < 1\%$) between the mean viscosity of mature green and red ripe tomato purees

7.2.1.1 Conclusion of the preliminary results

The amount of variation in puree stirred viscosity caused by the pureeing, the preparation process (blending, microwaving, diluting) and the viscosity measuring techniques (using the RVA) was acceptable ($cv < 10\%$). Natural variation between individual tomatoes doubles the error, this could have been due to each sample having to be diluted by a different amount to achieve the same solids, individual variation or that the second batch of tomatoes used were unlike those used for the bulk experiment.

However, when samples with expected differences in viscosity (as indicated by their maturity) are tested, then statistical differences can be seen. Therefore this method was concluded to be suitable for the viscosity screen. The dilution of the samples to the same solids content of 3.5% was adopted as standard for the rest of the introgression work.

7.2.2 Preparation of the tomato puree from the IL tomatoes

In total 76 Introgression lines with three plants per line, were raised from seed and grown in the glasshouse at Sutton Bonington Campus, under controlled conditions. This included the parent plants, the cultivated M82 and the wild type *S. pennellii*; their F1 progeny making up the IL library. Typically, three replicates (fruits) were harvested for 55 of the introgression lines. The remaining lines either not producing the required number of fruits or the size of the fruit was too small to assess. For example, the fruit from the wild type (*S. pennellii*) were not analysed due to the very small amount of pericarp tissue produced (typically less than 2g per fruit) and the fact these fruits remain green and very hard so it is difficult to define when they should be picked. The M82 fruit (the other parent) was considered to be the control sample of the experiment. Fruits from the lines were picked individually between May and July 2010. The samples were collected at the same stage of ripeness, defined as breaker plus seven days. The colour of the fruit once pureed was also assessed to verify whether the tomatoes could be defined as being at the same stage of ripeness as defined by the a^* value as well.

Tomatoes were sliced in half to remove the seeds and frozen within two hours of picking and then stored at -80°C. The subsequent preparation of the puree and analysis was conducted over a period of six weeks, due to the large number of samples. Frozen tomatoes were taken in a random order and puree prepared from each of these fruits.

The method for preparation of the puree is given in Section 3.2.6.2 and the procedure was as follows: tomatoes were peeled frozen and 40 g of pericarp was cut into approximately 1cm cubes and microwaved for 1 minute 20 seconds at 800W in a beaker, stirring half way through. The temperature was checked to be 95°C +/- 2°C. Samples were cooled to room temperature, and were blended with the Ultraturrax for 1 minute power 4. Solids content measurement was done on the day of puree preparation by calculating the percentage of loss on drying at 105°C for 18 hours of a 2 g aliquot of puree. Duplicate measurements were taken and the mean value used for the dilution of the puree to the same apparent solids content (3.5%), and the basis for this was to minimise the solids content being a major factor in the differences observed in viscosity and related attributes.

The remaining sample for each tomato was adjusted to 3.5% solids with distilled water. Stirred viscosity was measured on 25g of puree at 3.5% total solids at 180rpm for 10 minutes at 25°C. Purees were measured for stirred viscosity after 24 hours of its preparation.

Stirred viscosity (RVA), Brix, pH and colour measurements were assessed for the puree, then approximately 15g (equating to 15ml) was centrifuged for 1 hour at 2700 g at 25°C and the pellet to sample volume and weight ratios were calculated. The analyses undertaken for all the samples are listed in Table 7-3.

Table 7-3 Assessments, starting material and units method

Assessment	Starting material	units
Initial puree total solids	Undiluted puree	Oven drying aliquot of puree, Percentage
RVA stirred viscosity	Puree at 3.5% total solids	Stirred viscosity, Centipoise (cP)
Colour of puree (a*)	Puree at 3.5% total solids	Hunter a* value
Brix %	Puree at 3.5% total solids	% as sucrose
pH	Puree at 3.5% total solids	
Pellet/total weight sample ratio	Puree at 3.5% total solids	Ratio of pellet to total sample weight after centrifugation
Pellet/total volume ratio	Puree at 3.5% total solids	Ratio of pellet to total sample volume after centrifugation
Total solids of centrifuged serum	Puree at 3.5% total solids	Oven drying aliquot of serum after centrifugation, %
*Particle size	Puree at 3.5% total solids	Laser light scattering, μm

*some samples only

The results for the introgression line viscosity screen for 55 of the lines are presented and discussed below.

7.2.3 Assessment of the Introgression lines

Properties of the purees were measured at a consistent total solids (TS) content of 3.5%. These were; stirred viscosity, a* value (measurement of green to red colour) Brix, pH, pellet to sample volume and weights and serum total solids. The average (for all the 55 lines) plus the maximum and minimum mean value obtained within

the 55 lines tested for these assessments are shown in Table 7-4 to give an indication of the mean values and variation within the IL population. This table also shows the variations occurring within each of the IL lines by listing the average % coefficient of variation calculated from the 55 individual introgression line values assessed on typically three replicate samples of puree).

The grey shaded columns in Table 7-4 show the mean, standard deviation and coefficient of variation for the 55 samples measured. Large coefficients of variation were observed for some assessments, such as the stirred viscosity with a coefficient of variation of 34%. On each assessment, ANOVA was used to determine if there were statistical differences between the IL lines ($P < 0.05$), then Tukey's multiple comparison test with a confidence interval of 95% was used to determine how the tomatoes from the IL lines (categories) were grouped. The values for samples are shown in ranked order in bar charts in the following pages, and for ease of understanding the M82I and 5-1 are marked on each chart to identify variation in position of these lines. M82I represents one of the parent lines and 5-1 (contains wild type fragment in chromosome 5), were chosen as these samples occurred at different ends of the spectrum of results for stirred viscosity. These were marked on each chart as an indicator of how an individual line may vary in comparison with the rest of the samples.

Table 7-4 Assessments made on the IL tomatoes, with typically 3 replicate measurements (on 3 separate fruits) for each IL. A total of 55 lines were studied. ANOVA was used to determine if there were statistical differences between the IL lines ($P < 0.05$), then Tukey's multiple comparison test with a confidence interval of 95% was used to determine how the tomatoes from the IL lines (categories) were grouped

	Mean of the population			Range (IL with the lowest mean value and IL with the highest value)				Mean of 55 %CV	Statistical difference ANOVA at $p < 0.05$	Number of grouping in Tukey's Test
	Mean values \bar{x}	St dev. of 55 means	%CV	Minimum value	St dev. of the 3 reps	Maximum value	Sd dev. of the 3 reps	Average % variation (Coefficient of variation for the population)		
Initial total solids (%)	4.9	0.6	12.5	3.7	0.4	6.6	0.5	2.7	Yes < 0.0001	4
Stirred viscosity (cP)	132	45	33.7	71	7	283	36	25.1	Yes < 0.0001	6
a*	10.3	1.4	13.4	6.30	0.06	12.6	2.2	13.1	Yes < 0.0001	3
Brix (%)	3.0	0.2	7.3	3.40	0.25	2.48	0.40	8.5	No 0.024	1
pH at 25°C	4.1	0.1	3.3	3.76	0.15	4.38	0.21	3.2	Yes 0.00002	2
Precipitate : sample volume ratio	0.17	0.02	11.5	0.14	0.00	0.22	0.02	10.5	Yes < 0.0001	3
Precipitate : sample weight ratio	0.19	0.02	10.6	0.15	0.02	0.25	0.03	10.5	Yes < 0.0001	2
Serum total solids (%)	2.7	0.3	11.0	3.15	0.14	2.74	0.31	16.9	No 0.498	1
a* 2006 trial	17.2	3.7	21.6	6.7	8	23.8	1.1	x	x	x

7.2.3.1 Ripeness

Although viscosity was the most important measurement made on the puree, it was also necessary to establish the extent of the tomato ripeness at the time of harvest, in terms of colour. The possible relationships between this and viscosity have been discussed previously in chapter 6, and explain why the IL lines needed to be collected at the same ripeness. The ripening process of tomatoes and other fruits is a combination of many biochemical reactions, mainly observed by a change in colour and changes in the cell wall composition, causing softening, as discussed in section 2.3.

The tomatoes were collected at the same level of ripeness; (defined as breaker plus 7 days) and it was anticipated that there would be no significant difference in their colour. Therefore, it was assumed that any differences in viscosity or other attributes measured would not be caused by varying degrees of ripeness of the fruit. To verify this assumption, the mean a^* values of the pureed Introgression lines were evaluated, and these are shown in Figure 7-1. The mean values of individual ILs ranged from 6.3 to 12.6 with an overall mean (of all the IL results combined) of 10.3 a^* and the coefficient of variation for the population (all 55 lines) for a^* values was ~13%. ANOVA showed that there was a statistical difference in a^* values; the Tukey's test shows that the ILs has 3 groupings (a, b and c) that are statistically different from one another.

The tomatoes were grown in a glasshouse, lit with a mixture of artificial and natural light, and the plants located in a randomised order so that the conditions during the growing period would not influence the ripeness or other attributes of the fruit.

The observed differences in a^* values (colour) could have been due to several reasons or combinations of reasons. For example, some of the ILs have different fruit colour and carotenoid-content phenotypes. Figure 2-18 (Zamir, 2001) shows six introgression lines (ILs) that demonstrate this. For example, IL 10-1 shows a mixture of green and red even though it is a mature fruit. Once pureed, any variation observed within a single fruit would be minimised, but could explain why IL lines vary from one another. None of the ILs shown in this photo were shown to be very different in this study, perhaps due to the fact that the b^* (blue to yellow) value is needed (or a ratio between the a^* and b^*) to detect yellowness.

The one notable point was that in this set of data both 12-3 and 12-3-1 had an introgressed area of DNA in common with each other, and they both have a^* values lower than the majority of the other IL lines. This could suggest that the colour could be affected by genes located on this part of chromosome 12. It is possible that some samples had not matured to the same level and/or the samples all did not have the same colour even when matured.

The mean a^* value of the IL tomatoes can be compared with the commercially grown tomatoes discussed in chapter 4; the red ripe had a mean a^* value of 19.8

(2.6), and the pink (less ripe) tomatoes 7.6 (1.9) (shown in Table 4-1). There were slight differences in the testing protocol between the IL lines and the commercial tomatoes, which were tested on their skin at 3 different positions on the fruit. This difference between the colour of the commercial and IL tomatoes could suggest that the introgression lines were not as ripe as the red ripe commercial tomatoes, or some difference was due to the fact that the IL a^* value was measured on the puree without the skin, and the pericarp tissue was less red than the skin.

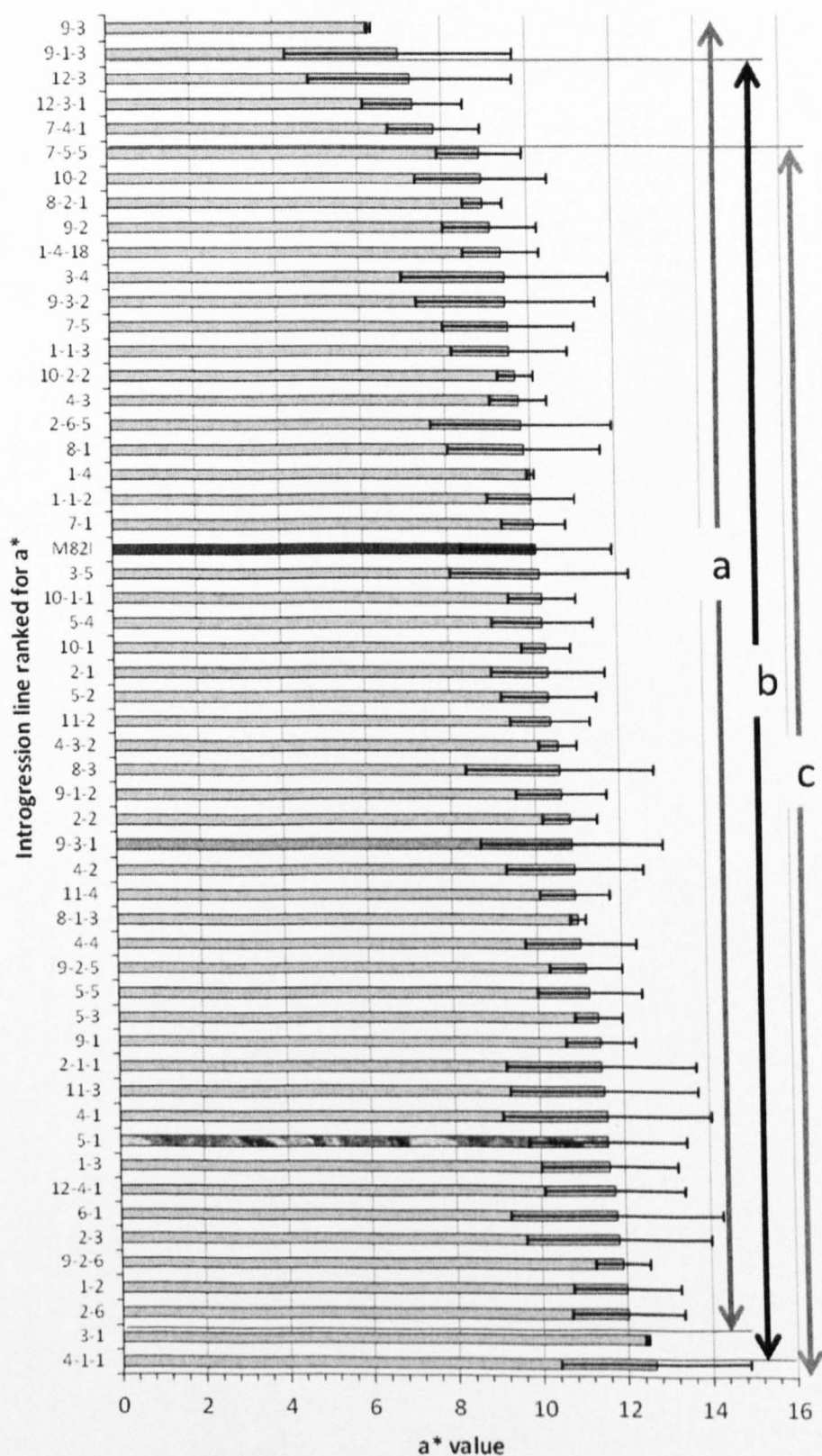


Figure 7-1 Introgression lines ranked by a^* measured on the puree at 3.5% TS
 ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

7.2.3.2 Viscosity and associated attributes

Having assessed that the tomatoes were at the same stage of ripeness, as shown by the three Tukey's groupings for a* overlapping to a large extent, shown in Figure 7-1), the mean stirred viscosity of each of the introgression lines were compared, and results displayed in Figure 7-2. The mean value was calculated from typically three replicate measurements on three individual fruits per line. When ranked for viscosity in this way, the most notable feature is the variation in viscosity values as measured for the three replicate samples from the same introgression line; it would seem that the variation between individuals could be greater than that between genetic variations, indicated by the large error bars. The control M82I had a relatively high viscosity of 189cP, compared to the mean viscosity of 133cP for the 55 ILs. Despite the large standard deviations between replicates of the same introgression line, there is a statistical difference between the IL lines, as determined by ANOVA. The less viscous ILs had mean stirred viscosity values of approximately 90cP and the most viscous (such as 2-1 and 3-4) with mean values of over 250cP. Tukey's test differentiated 5 groupings, (a) to (e), Figure 7-2. The Tukey's groupings overlap to a large extent.

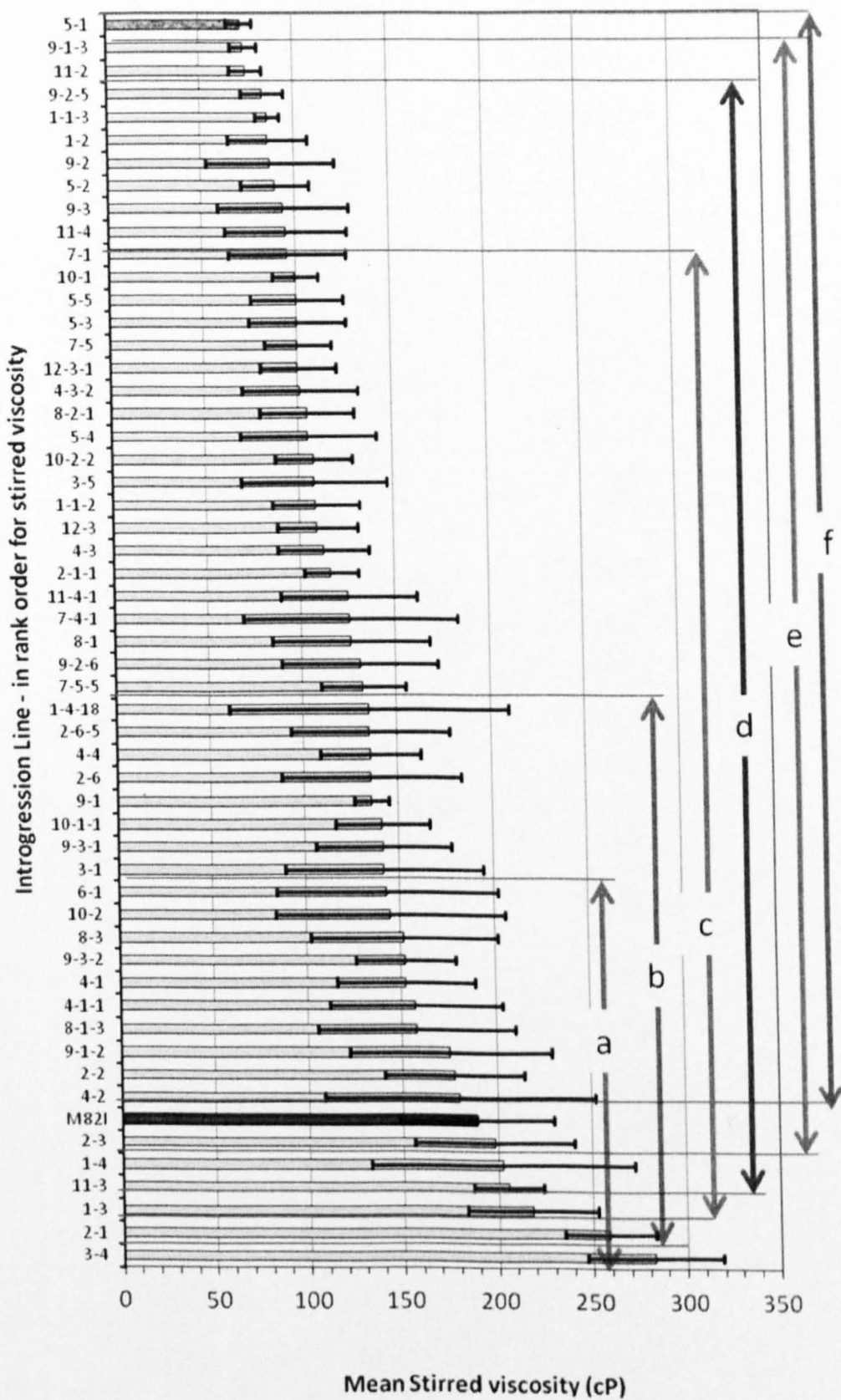


Figure 7-2 Introgression lines ranked by stirred viscosity measured on the puree at 3.5% TS
ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

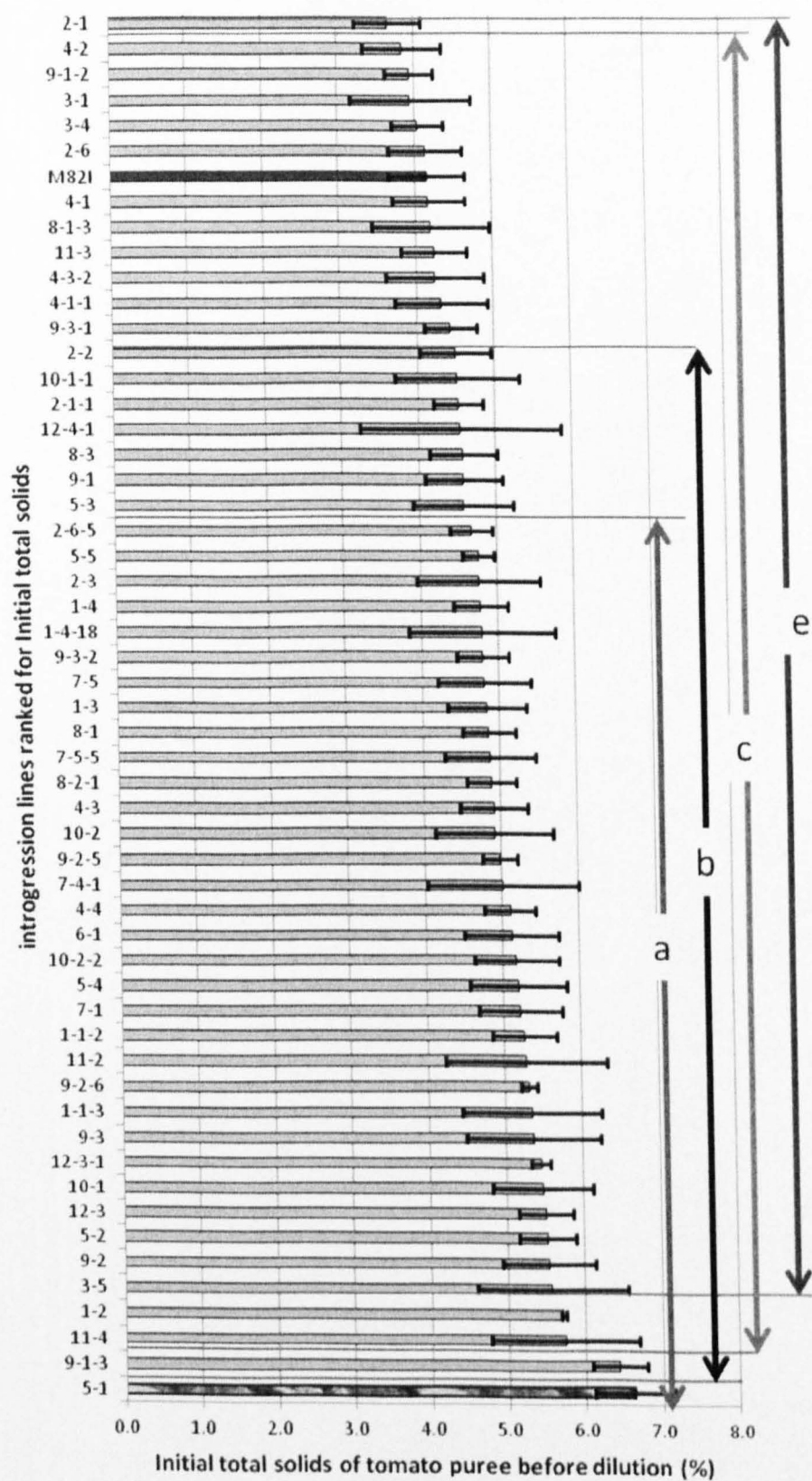


Figure 7-3 Introgression lines ranked by percentage of initial total solids measured on the puree before dilution to 3.5% total solids ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

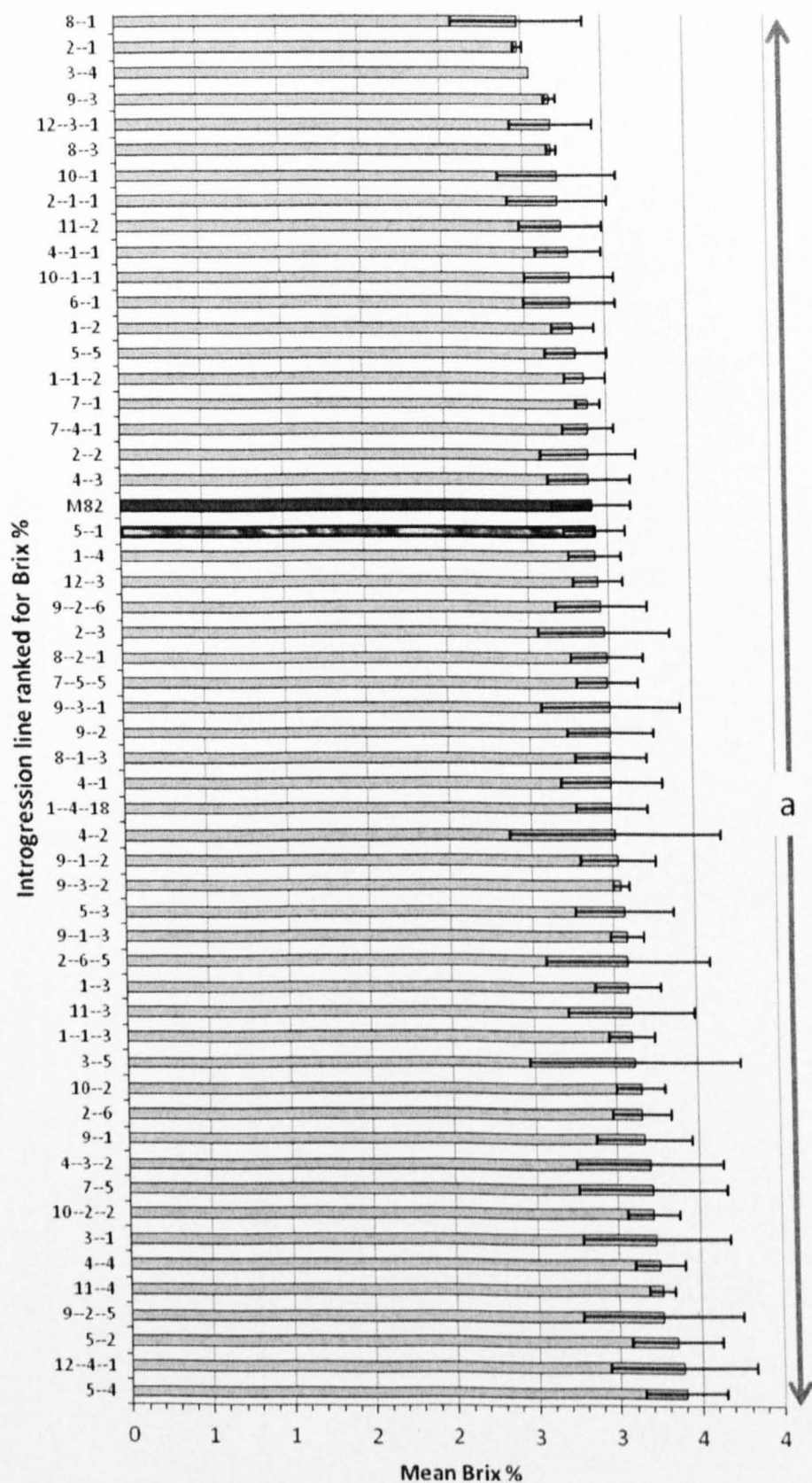


Figure 7-4 Introgression lines ranked by % Brix measured on the puree at 3.5% TS
ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$ Tukey's multiple comparison test)

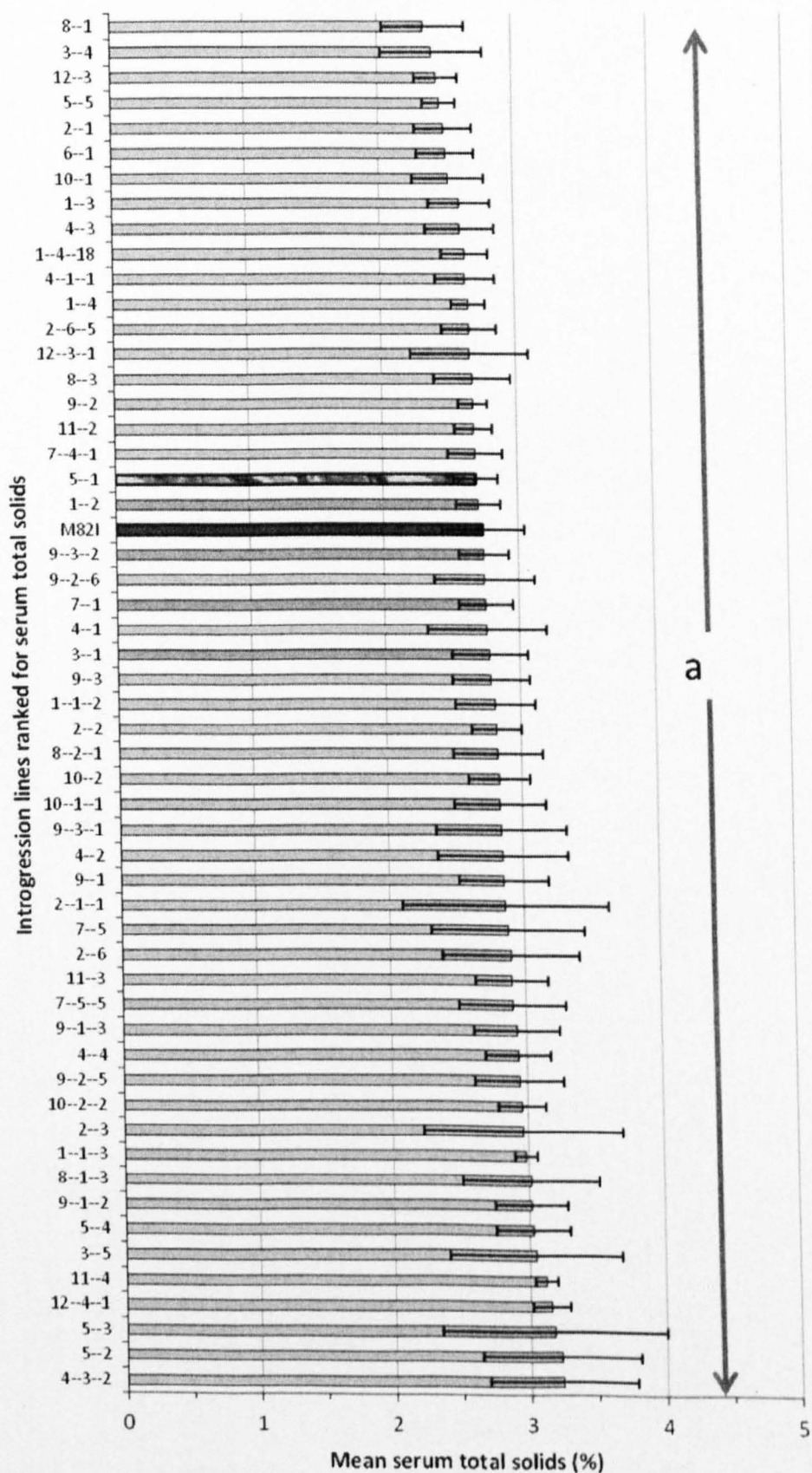


Figure 7-5 Introgression lines ranked by serum total solids measured on the puree at 3.5% TS
ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

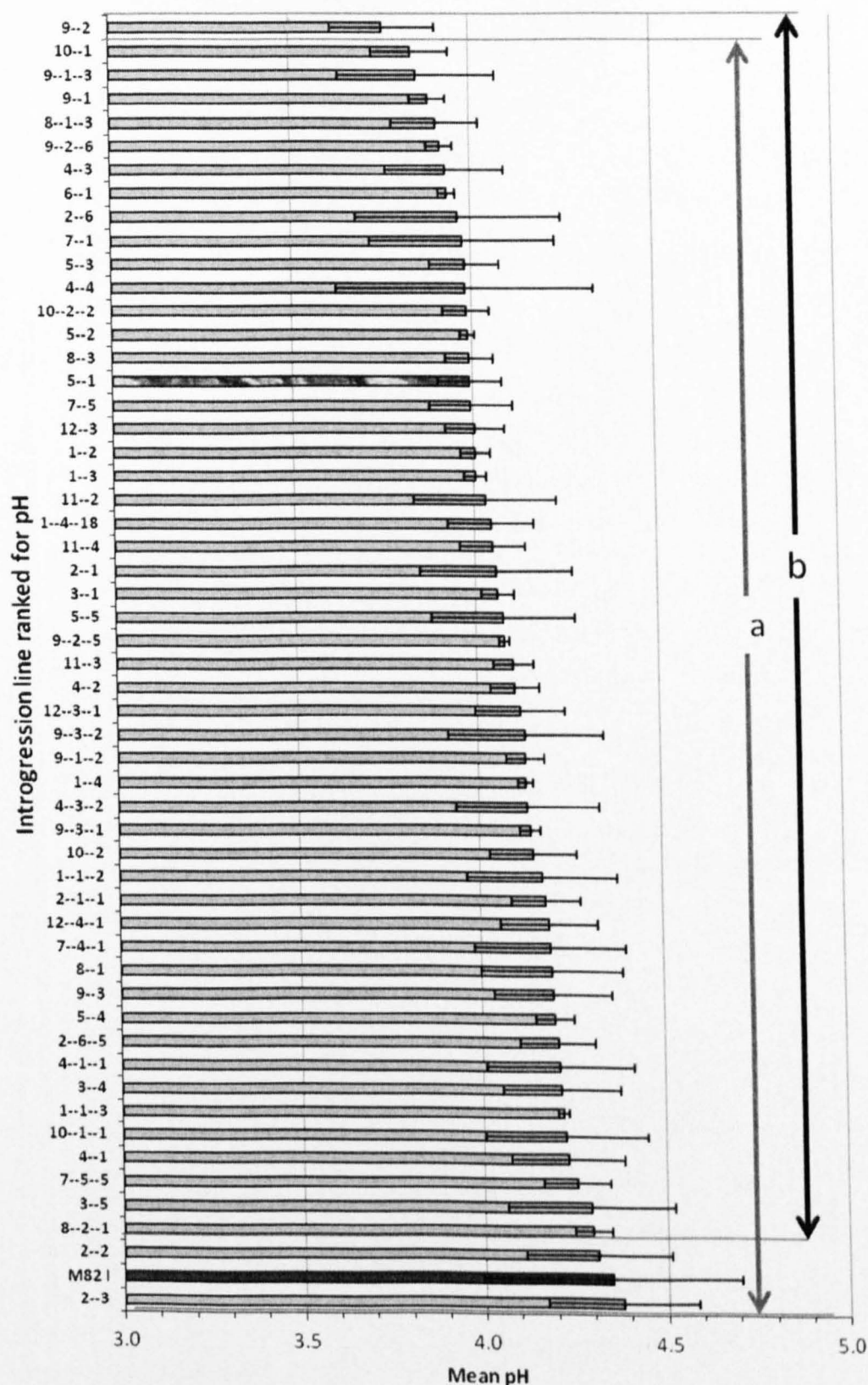


Figure 7-6 Introgression lines ranked by pH measured on the puree at 3.5% TS
ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

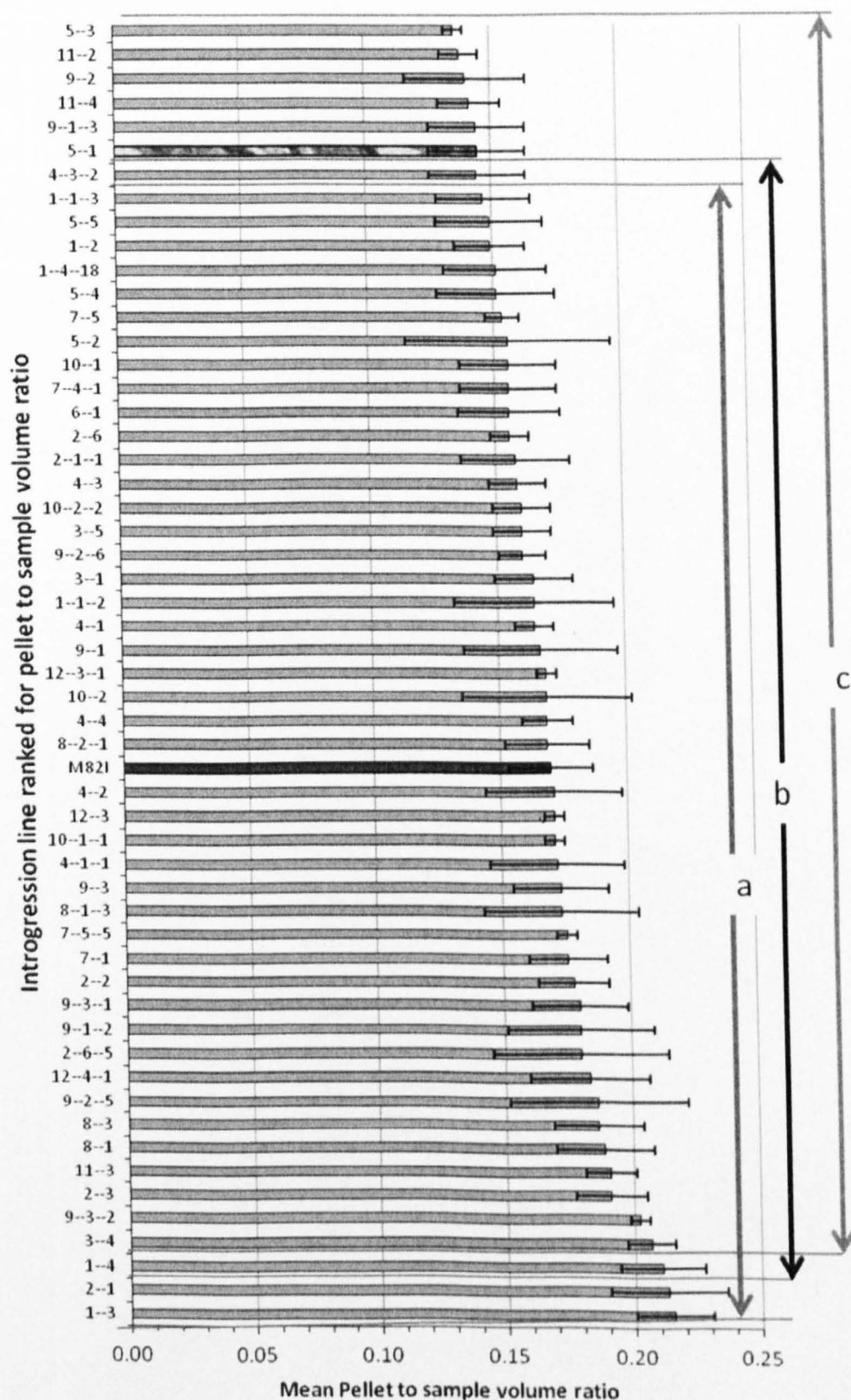


Figure 7-7 Introgression lines ranked by pellet to sample volume (particle to total sample volume) ratio measured on the puree at 3.5% TS. ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

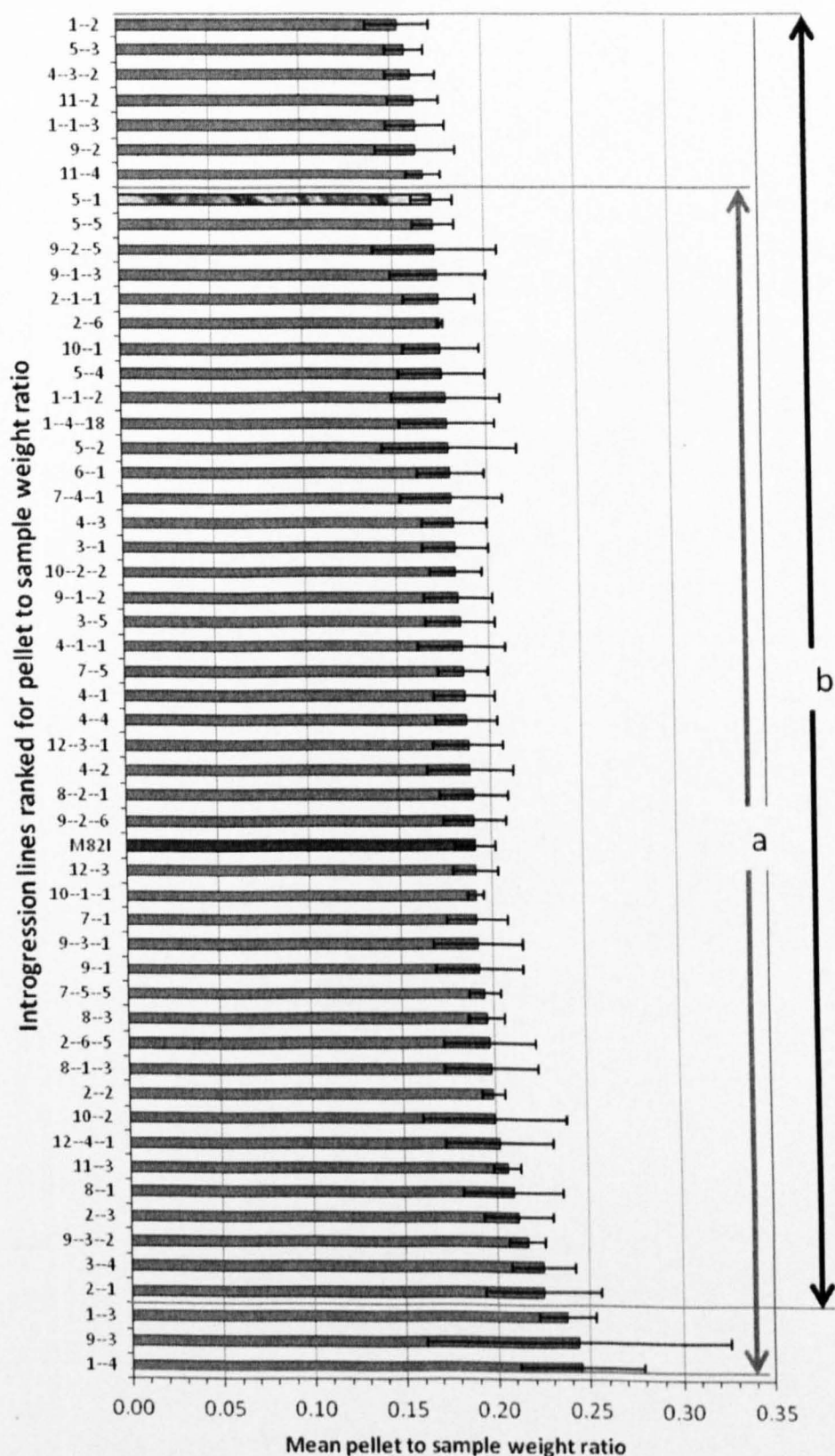


Figure 7-8 Introgression lines ranked by pellet to sample weight (particle to total sample weight) ratio measured on the puree at 3.5% TS. ILs with different and non-overlapping ranges of letters are significantly different ($p < 0.05$; Tukey's multiple comparison test)

The chosen ILs marked on the histograms (Figure 7-1 to Figure 7-8) indicate M82I (a control) and IL5-1. IL 5-1 has the lowest viscosity, whereas in comparison M82I has a high viscosity when compared to the other lines. These samples, likewise rank at opposite ends of the values for other parameters, for example in colour in Figure 7-1 (a^*) and pH, Figure 7-6. However, a plot of these assessments against stirred viscosity values (see Figure 7-9 and Figure 7-10) show no correlation between the parameters and therefore differences in the colour and pH do not seem to be relevant to the stirred viscosity. Although the samples sit at each end of the distribution values for colour and pH they are in fact encapsulated within the same statistical groups.

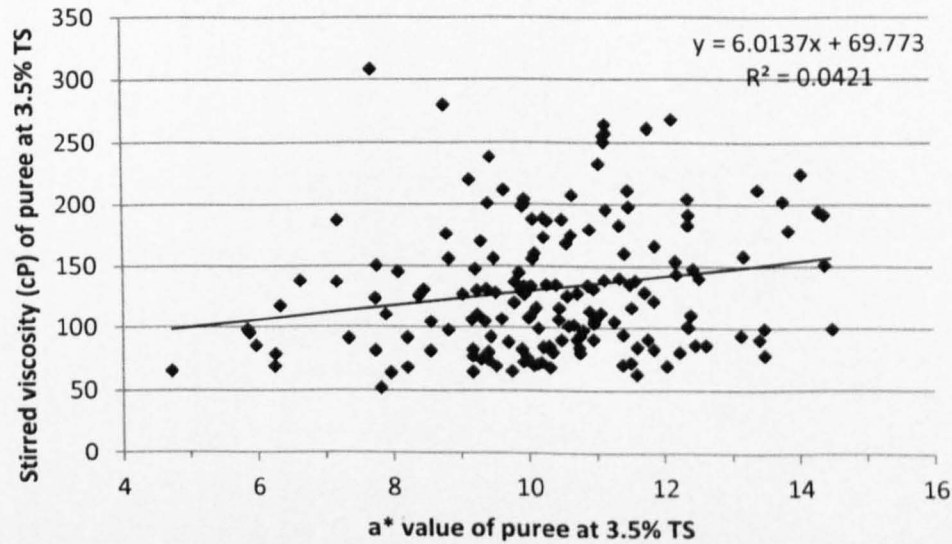


Figure 7-9 Stirred viscosity of puree at 3.5% total solids as a function of a^* colour value. (individual values of 3 fruits per line, for 55 lines) the two samples that are furthest from the line are: IL3-4b and 2-1b).

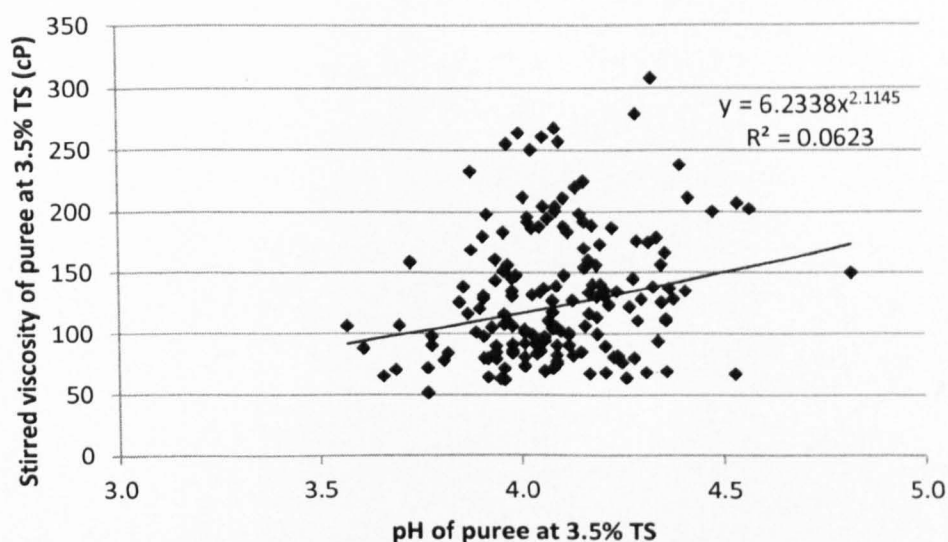


Figure 7-10 Stirred viscosity of puree at 3.5% total solids as a function of pH. (Individual values of 3 fruits per line, for 55 lines)

For the serum total solids both the marker lines (M82I and 5-1) are at the higher end of the distribution when placed in rank order for serum total solids (see Figure 7-5), while for Brix they sit as neighbours on the histogram (see Figure 7-4). Using all the values measured on a scatter diagram for these attributes versus stirred viscosity shows no correlation between either of these measures and the stirred viscosity (Figure 7-11 and Figure 7-12).

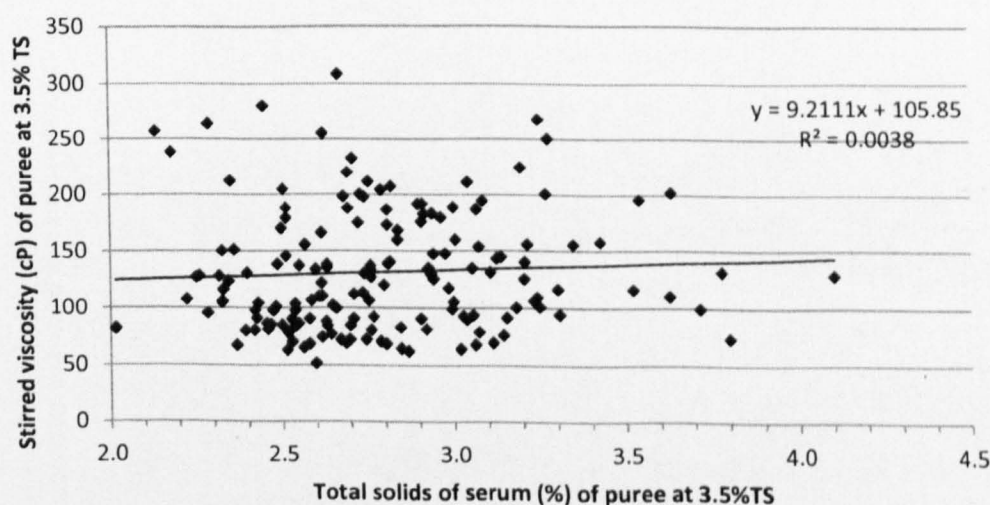


Figure 7-11 Stirred viscosity of puree at 3.5% total solids as a function of serum total solids. (Individual values of 3 fruits per line, for 55 lines)

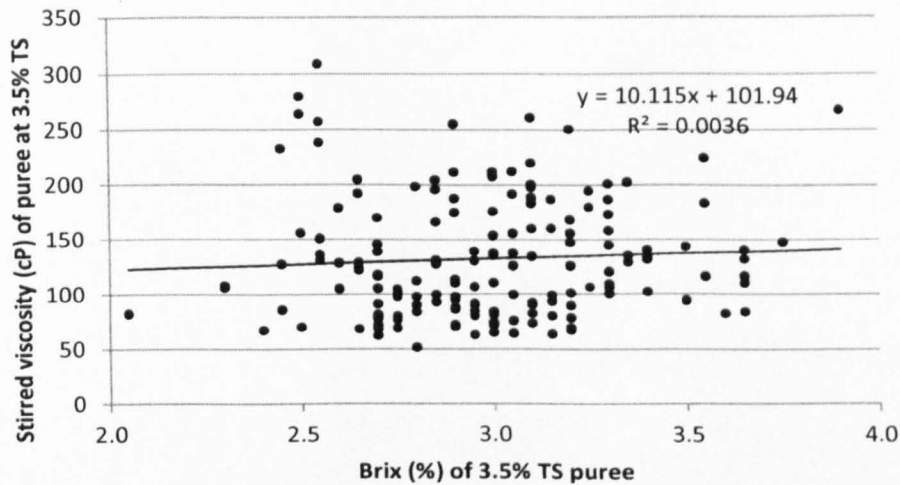


Figure 7-12 Stirred viscosity of puree at 3.5% total solids as a function of Brix value. (Individual values of 3 fruits per line, for 55 lines)

It therefore can be concluded that the variation in colour, pH, serum total solids and Brix of the samples when diluted to 3.5% solids have no direct relationship with the stirred viscosity. It should be noted the ranges for these values (a*, pH serum total solids, Brix) shown by individual introgression lines is low in comparison to the variation between the individual fruits.

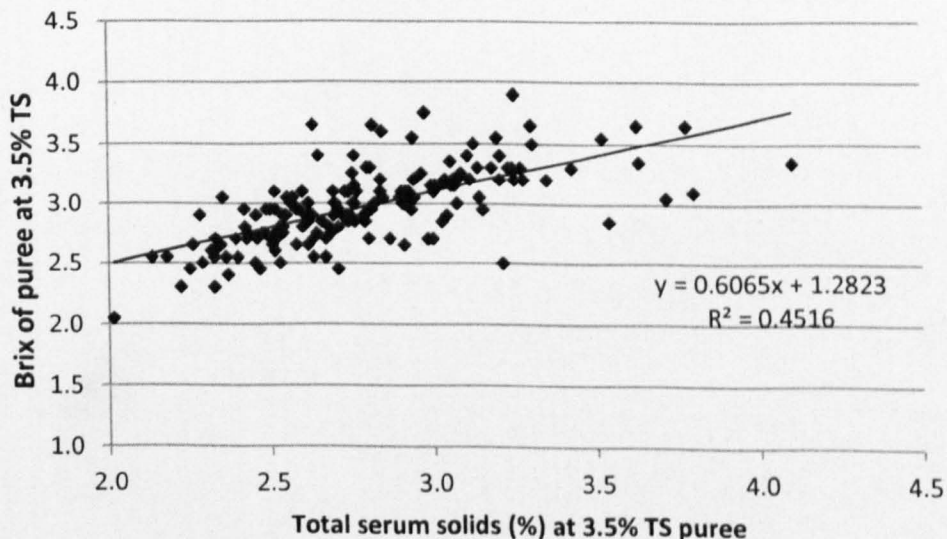


Figure 7-13 Brix of puree as a function of serum total solids on puree diluted to 3.5%TS. (Individual values of 3 fruits per line, for 55 lines)

Figure 7-13 shows the relationship between the values for Brix and the total serum solids. The data indicated that the probability of the solid values and Brix are not correlated at less than 0.1% (significance of correlation coefficient). It would be expected that these values would be correlated, but the Brix values were expected to be lower than the serum total solids and this was not the case. The samples showed Brix values from just over 2 to 4% despite the total solids being adjusted to 3.5%. The higher values obtained for the refractive index values are obviously in doubt. A feature of the viscosity values is that all samples were diluted to the same concentration, as it is well known that concentration is an important factor in viscosity (see section 2.5.2). What was therefore unexpected was that the total solids of the original puree was so well correlated ($R^2 = 0.58$ and probability of these values not be correlated was $<0.1\%$) with the stirred viscosity when diluted to 3.5% TS, Figure 7-14. The samples that lay furthest away from the trend line come from different replicates and there is no simple relationship between these non-conforming samples. The question then arises, what is the difference within the total solids that impacts on the viscosity? For this complex sample matrix the solids can be considered as those in solution (soluble material) and those in particle form (insoluble). A plot of total solids of the puree against soluble solids in the aqueous phase shows little correlation, as shown in Figure 7-15. This indicates that diluting the samples to the same overall concentration (3.5%) renders *all* the solids in the sera to the same value.

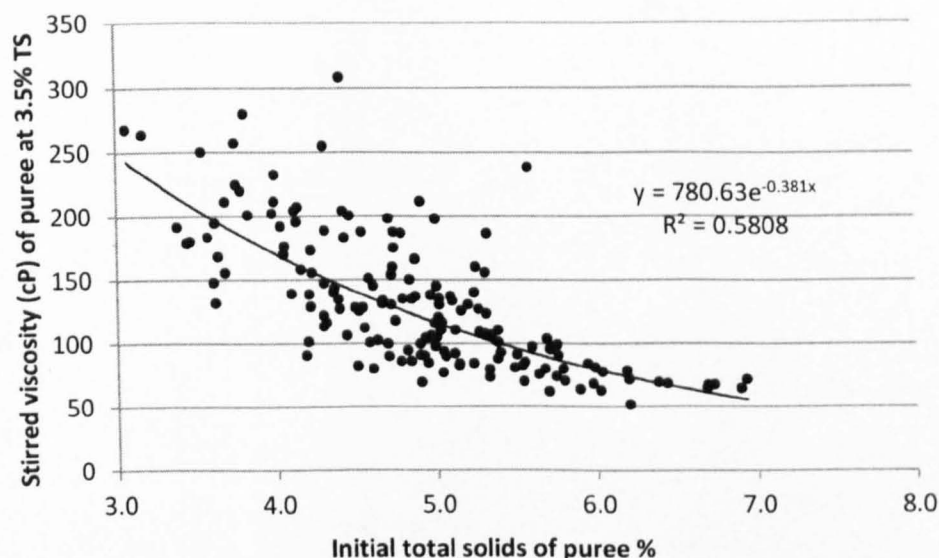


Figure 7-14 Stirred viscosity of puree at 3.5% total solids as a function the original total solids of the puree (individual values of 3 fruits per line, for 55 lines).

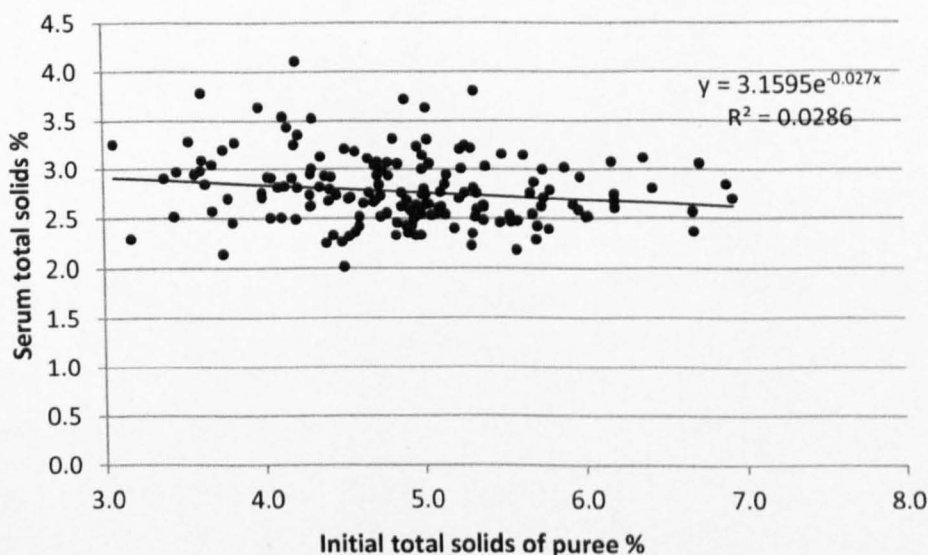


Figure 7-15 Serum total solids (after puree had been diluted to 3.5% TS) as a function the original total solids of the puree (individual values of 3 fruits per line, for 55 lines).

If the variation in the samples is not from the soluble solids it should be affected by the particle fraction. This was assessed by the ratio by weight or volume of the particle (pellet) phase after centrifuging the puree at 3.5%TS (see methods section 3.2.11). Figure 7-16 shows that high amounts of particle (pelletable material) typically give rise to high viscosity (probability of not correlating <0.1%. Figure 7-17 shows the correlation between the particle phase volume compared to the original total solids of the puree. Although the R^2 value does not seem high (0.16), there

are 164 data points and the probability of correlation is calculated to be >99.9% (see statistical analysis section 3.2.13.2 for test for the significance of the Pearson product-moment correlation coefficient). Low original total solids gives rise to high levels of particles (expressed as volume). The variations in the stirred viscosity should have been apparent by diluting all the samples to the same overall concentration.

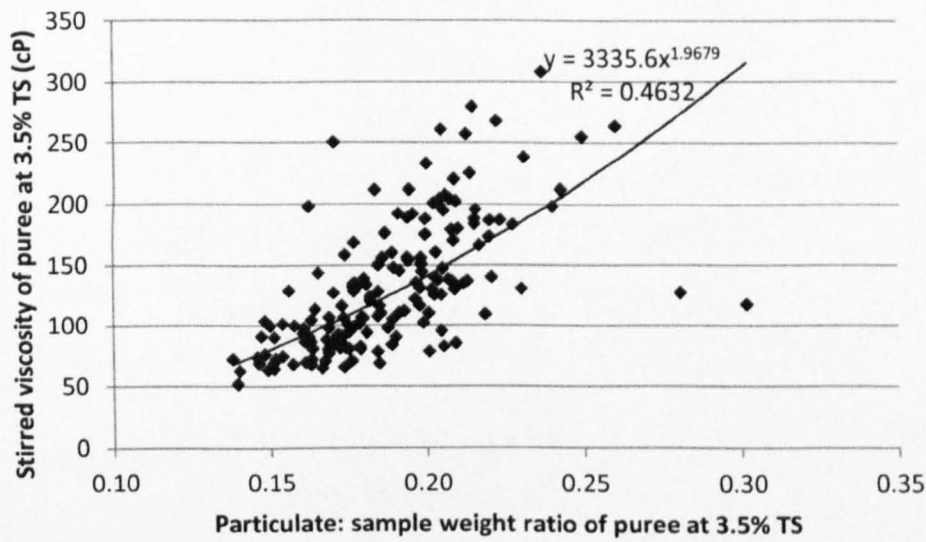


Figure 7-16 Stirred viscosity of Introgression lines as a function of the pellet (particle): sample weight ratio at 3.5% TS. (Individual values of 3 fruits per line, for 55 lines).
 [The four samples that are furthest from the line are single representative samples of: 4-2a; 6-1c; 1-4b, 9-3a.]

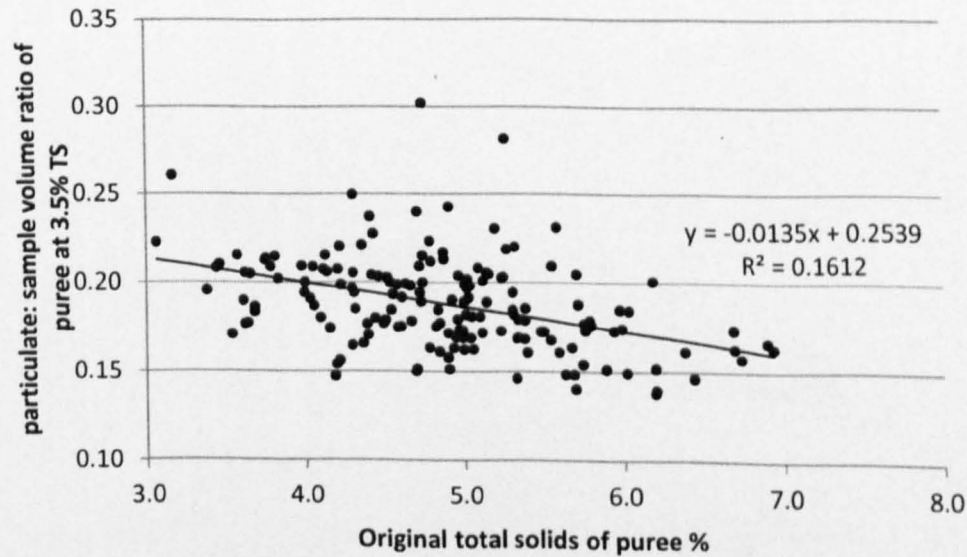


Figure 7-17 Ratio of particle to sample volume of puree diluted to 3.5% TS as a function of the original total solids of the puree (individual values of 3 fruits per line, for 55 lines)

7.2.4 Analyses of data from IL screen

Section 7.2.3 shows the data collected from the Introgression lines and it was expected that simple relationships between some of these parameters would have been clear. A feature is that there is much fruit to fruit variation and therefore this may cloud some correlations. To look at all the data, including the contribution from individual fruits a statistical procedure was used (described in the methods chapter, section 3.2.13). Multivariate principal component analysis (PCA) was used for statistical analysis on the assessment data for the introgression lines. This was done to obtain an overview of the way in which the different physical properties of the tomato purees are interrelated.

The biplot from this analysis is shown in Figure 7-18. The first and second principle component (PC1 and PC2) explain 37 and 25% of the variation, respectively. Thus when reading the interpretation below, it must be remembered that 38% of the variation remained unexplained.

From the PCA plot it can be seen that the initial total solids (written as total solids on the PCA plot) of the puree had a negative correlation to viscosity (Viscosity) and ratios of the pellet to sample weight (weight) and volume (volume), shown across PC1. (Remember that these measurements were done on purees diluted to 3.5% total solids). This positive correlation between pellet weight, volume and stirred viscosity observed in this data for the introgression lines was also shown in data

presented in the chapter, section 6.2.4, on one sample of red ripe puree. This suggested that this positive correlation was not due to experimental error.

PC1 could be thought of as giving some understanding for the behaviour of the puree, whereas PC2 could be seen as explaining the eating quality of the tomatoes, with Brix (sugar content, sweetness) serum solids, and colour being positively correlated. The eating qualities of Brix, serum solids and colour were also shown to be negatively correlated with pH on PC2, which could be due to the more ripened fruit being sweeter, and less acidic. It would seem a logical conclusion to determine that in general, ripened whole fruit are less acidic (higher pH) and sweeter (higher Brix) and that some degradation of the cell walls could have occurred to give a higher serum solids concentration, and this has been shown by previous research. Despite the fruits being harvested at the same age, 7 days after the breaker stage, the PC2 attributes shows that there were slight variations between the ripeness of the tomatoes harvested. The other main point to observe is that there is a large spread in the replication of data points; for example in Figure 7-18 the four replicates of the control M82I are distributed over a relatively large area on the plot, and are ringed to make them easier for the reader to view. The main conclusion of the data displayed on the PCA bi-plot Figure 7-18 therefore was that it was very difficult to use the data to identify the genetic basis for effect on viscosity, due to the variation between the replicates.

However, even with the variability in the replicates discussed above, the critical factors for viscosity were shown to be that;

- i. viscosity decreased with original total solids content (samples with high initial total solids had the lowest viscosity)
- ii. viscosity increased with the pellet to weight and volume ratios

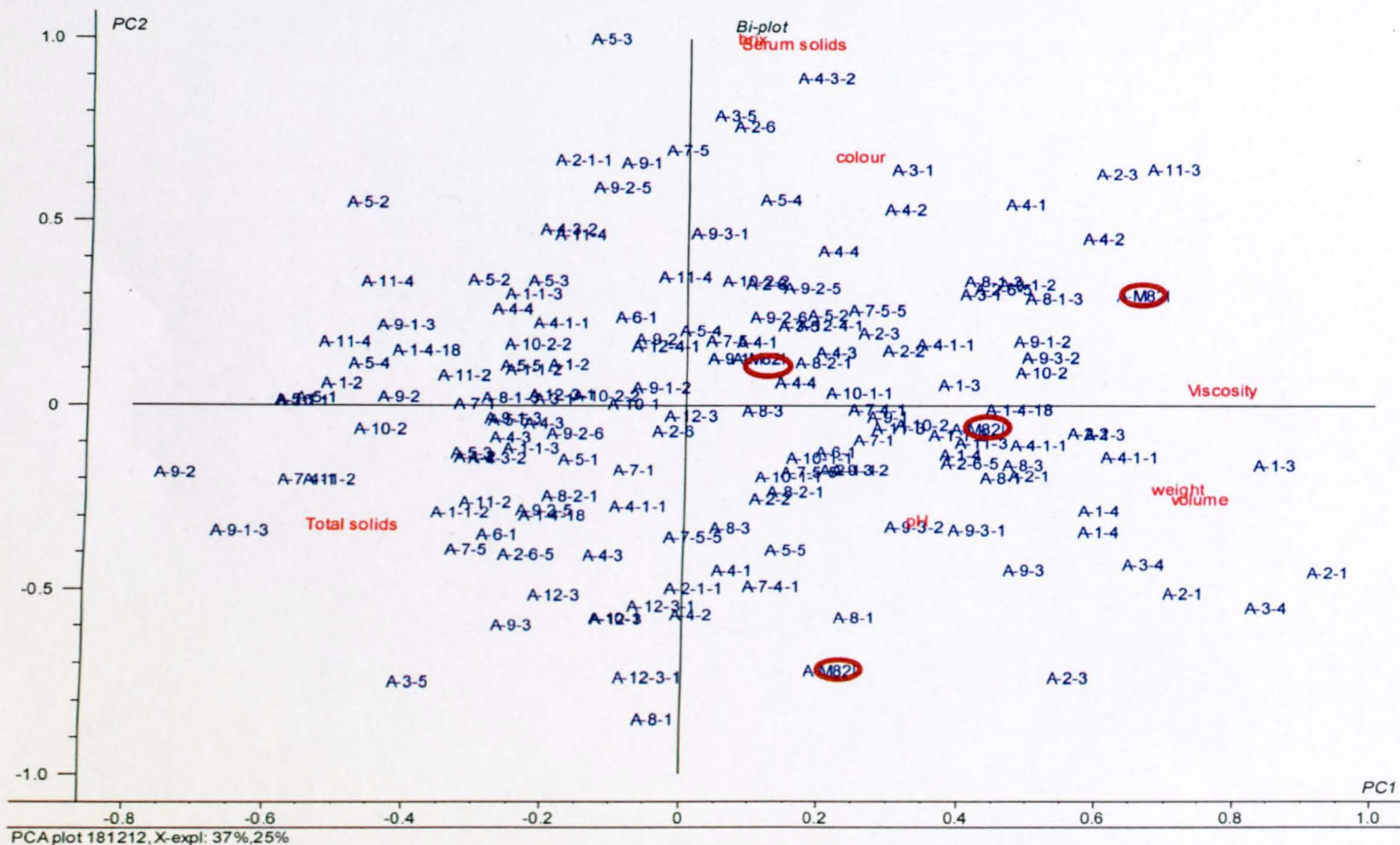


Figure 7-18 PCA bi-plot of viscosity screen for typically 3 replicates of IL tomato puree samples measured for stirred viscosity at 3.5%TS. Key – Total solids = initial total solids, pH= pH, weight = pellet to sample weight ratio, volume = pellet to sample volume ratio, Viscosity = stirred viscosity, colour = a* value, serum solids = serum total solids, Brix = Brix.

As explained most of the viscosity data was measured on the diluted samples, but a few samples were measured before dilution. The viscosity of these samples and the viscosity of the samples diluted to 3.5%TS were plotted against the original solid contents are indicated in Figure 7-19. A reasonable correlation ($R^2 = 0.466$ ($p > 0.998$ that the values correlate) is shown for the diluted samples and this confirms the early findings when all the replicates from the 55 IL lines were studied (Figure 7-14) and indicated that when diluted to the same solids content the viscosity was lower for the samples with the highest initial total solids (as they needed more water to dilute them). It would be expected that the higher the solids the greater the viscosity and there was a positive correlation between the original solid contents and the viscosity of the undiluted puree. The correlation was weaker ($p > 0.96$) when compared to the undiluted samples; this appears to be due to the wide range of viscosity values for the higher solid content samples. The fact that total solids was not a main contributor to the stirred viscosity was observed for the tomatoes at different stages of ripeness, results shown in Table 6-1 in the previous chapter. Figure 7-20 shows the same viscosity data from the puree before dilution against the data for the same diluted samples. The data seems to indicate that there is little correlation between the viscosity of the undiluted purees and the viscosity after dilution to the same solids content.

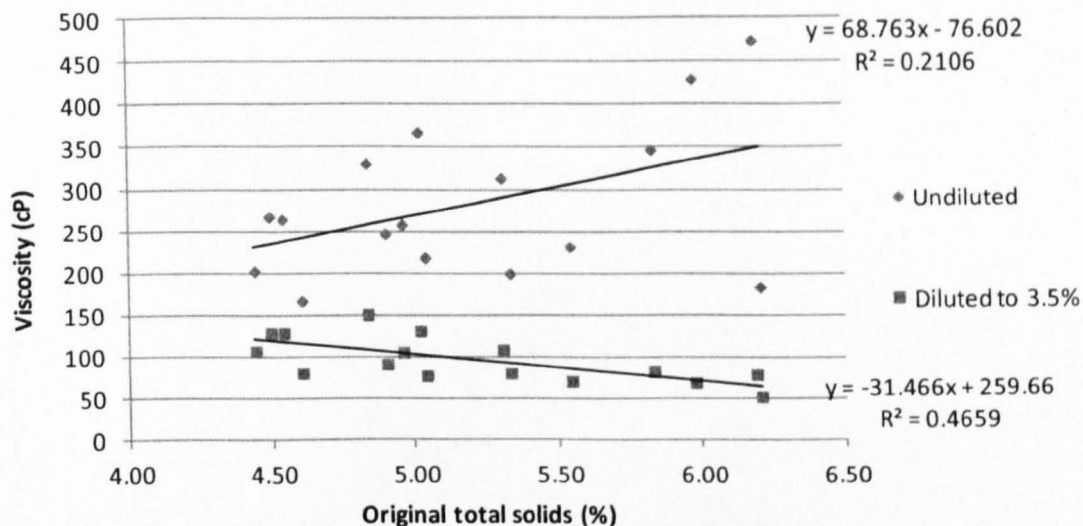


Figure 7-19 Comparison of stirred viscosity values at the puree original total solids and when diluted to 3.5%TS plotted against the original solid content, on a restricted number of tomato fruits from the IL lines.

[Nb, data points include 9-3 and 10-3 IL lines that are not included in other parts of the data. 9-1-3 initial visc of 474, (and was diluted to 3.1% ts) and 9-3.]

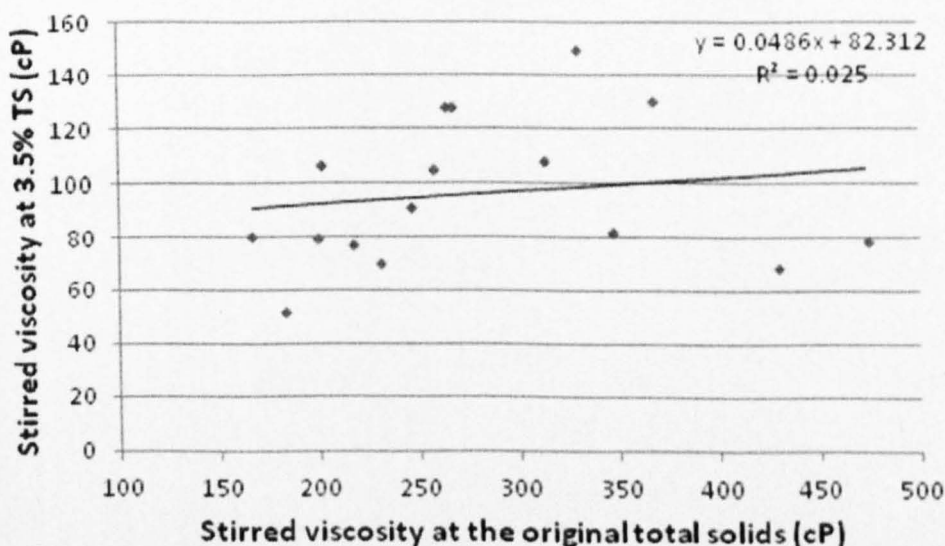


Figure 7-20 Comparison of stirred viscosity values of a restricted number if tomato fruits at the puree original total solids and when diluted to 3.5%TS.

[Nb, data points include 9-3 (183,52) and 10-3 (347,82), IL lines that are not included in other parts of the data. 9-1-3 initial visc of 474, 79 (and was diluted to 3.1% ts) and 9-3 430, 68]

The PCA shown in Figure 7-21 shows the combined analyses data for the restricted set of IL samples where the viscosity was measured for the undiluted sample. The plot of PC1 and PC2 combined represents 73% of the data. There appears to be a group of values (located in the bottom right of the PCA plot), plus four samples that do not conform to this major grouping. Investigation of the individual data sets do

not indicate clear reasons why these samples are different, so it seems that they may vary across a range of parameters, this could well be the case as the samples are genetically different.

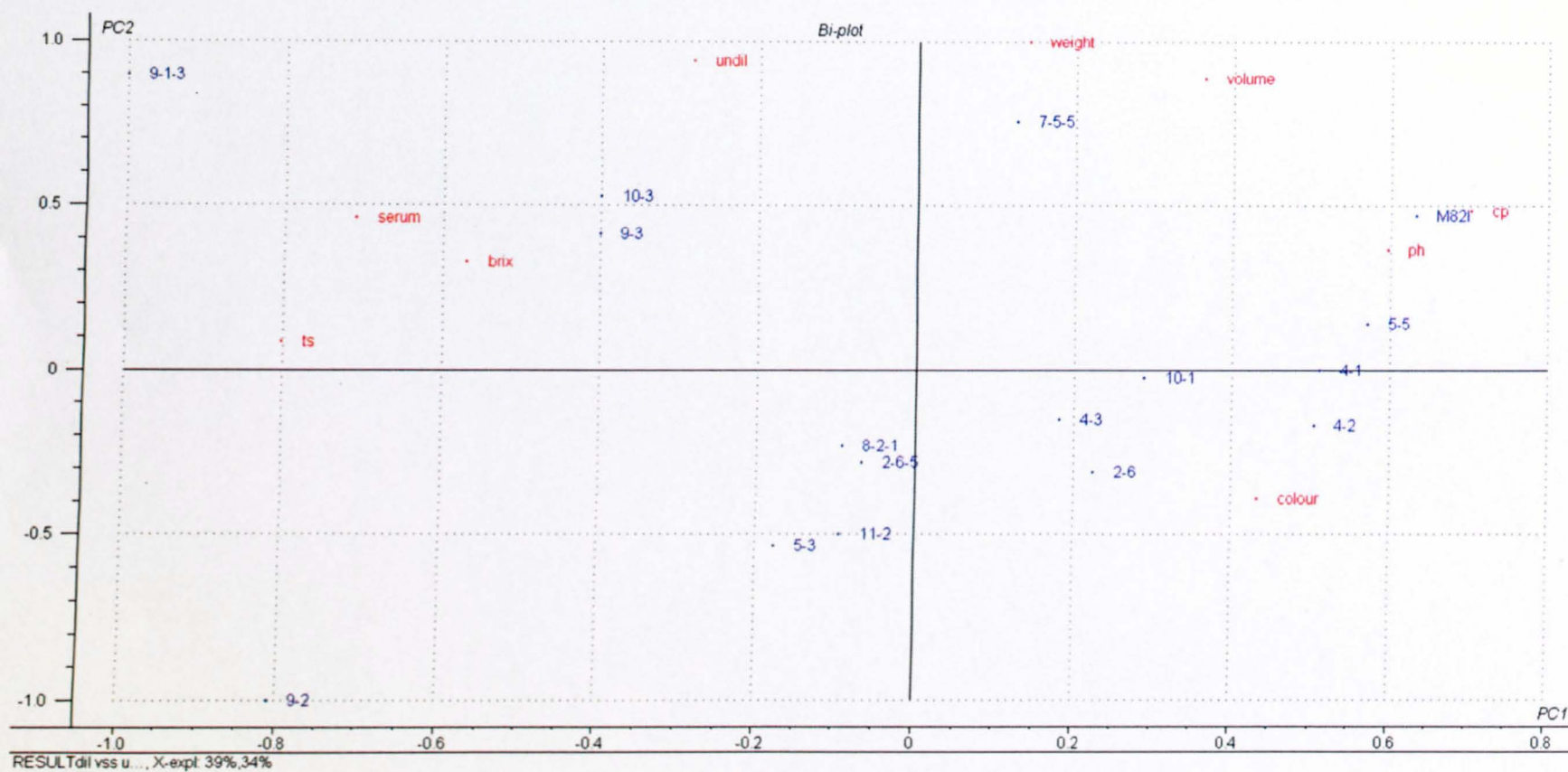


Figure 7-21 PCA bi-plot of viscosity screen for a selection of IL tomato puree samples measured for stirred viscosity at original total solids and diluted to 3.5%TS. Key - ts = initial total solids, ph= pH, weight = pellet to sample weight ratio, volume= pellet to sample volume ratio, undil = stirred viscosity at initial total solids, cp = stirred viscosity at 3.5% TS, colour = a* value, serum = serum total solids, Brix = Brix

7.2.5 Factors affecting viscosity of purees at the same solids content

There was a strong indication from the IL samples that the higher the original total solids, the lower the viscosity values were when diluted for the viscosity measurements. A hypothesis to explain this could be that the tomato puree with the higher total solids contained materials with a lower hydrodynamic volume, compared with the purees with the lower original total solids. This returns us to the question 'What sort of material gives rise to the differences in the hydrodynamic volume?' For the introgression lines, and as shown in Figure 7-12 there was no correlation between the Brix value and the viscosity of the puree when diluted to a constant solids level of 3.5%.

The pellet (particle) to sample weight ratio for puree at 3.5% total solids made from the introgression lines was determined by centrifuging puree at 2700g for 1 hour at 25°C, and calculating the ratio of the pellet weight (after pouring off the serum) to the weight of the serum and puree. As the data presented in Figure 7-16 shows, the pellet: sample weight ratio varied from 0.14-0.30 with good correlation with stirred viscosity of the puree. From previous chapters, it was found that viscosity had some correlation with the pellet (particle) to sample weight ratio (see section 6.2.4), whereby adding more of the particle fraction increased viscosity. Other researchers have found similar correlations (Takada and Nelson, 1983, Tanglerpaibul and Rao, 1987).

Although for these current studies as indicated in this section for one stage of

ripeness, and in section 6.2.4 for a range of ripeness stages, the change in particle fraction did not completely reflect the observed viscosities. However, it is clear that the pellet weight at constant solids content of the different tomatoes was not the only factor affecting stirred viscosity.

Another possible factor affecting viscosity of fruits at the same stage of ripeness was the size of the particles. This was measured by light scattering (see methods section 3.2.9) on a small number of the samples. Figure 7-22 shows that there was no simple correlation between particle size and stirred viscosity. Although the R^2 value does not seem high (0.12), there are 51 data points and the probability of correlation is calculated to be >98%. This suggests that the particle size could be a relatively important factor in tomato viscosity.

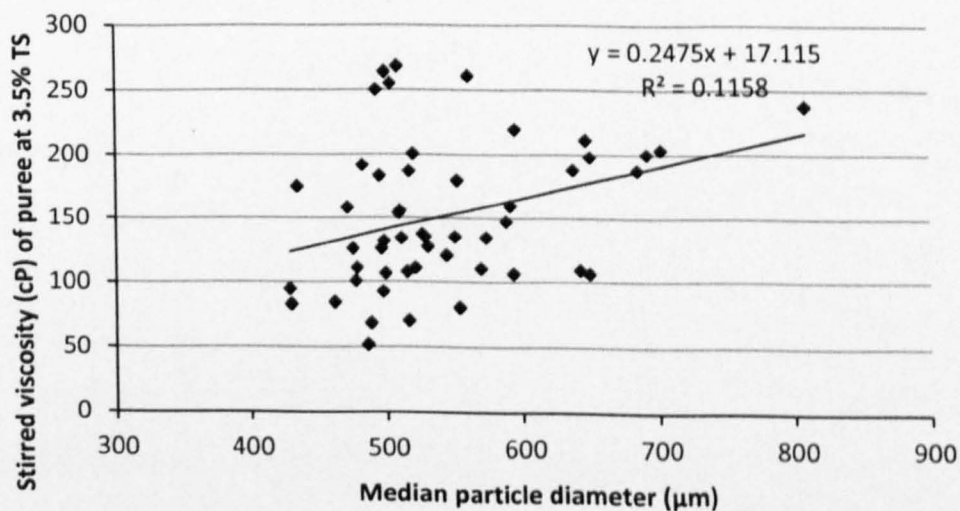


Figure 7-22 Stirred viscosity of tomato puree from the IL population as a function of median particle diameter, measured by laser light scattering.

7.2.6 Viscosity and texture

The decision to work on the IL lines was made with the knowledge that the genetic variation impacted on fruit texture. For example Chapman et al (2012) investigated ILs 2-3 and 2-4, two lines with firm fruits. Therefore the attributes of the tomato puree at 3.5% total solids were compared with pericarp texture and fruit colour data from previous studies on the introgression lines, conducted by Dr Poole and co-workers at the University of Nottingham in 2006. In the 2006 experiment, the texture of the pericarp expressed as mean Maximum load, N and was obtained on three replicates (3 fruit per line) using the same experimental method as used in 3.2.4, and the fruit were grown under the same glass house conditions and collected at the same stage of ripeness (B+7). This comparison with the previous set of data was done here to determine whether the texture of the fruit affected the quality of the puree or just the bulk fruit texture and therefore the eating quality of the fruit. Some of ILs from the total population (of over 70 lines) had to be eliminated from the analysis as texture data was not available for a small number of lines that had been included in the current viscosity screen.

An overview of the way in which texture of the fruit and puree attributes are interrelated is shown with a PCA biplot, Figure 7-23. The mean values of the (3 replicates) per introgression line were used for each of the variables. PC1 and PC2 combined explain 50% of the variation. It should be remembered that the data are a combination of analyses from different crops. The first

principal component (PC1) was found to explain 33% of the variation. As shown previously in Figure 7-18, the main driving forces in PC1 were stirred viscosity (cp) and pellet weight and volume ratios which were all positively correlated. The eating qualities are grouped on PC2, ie sugar (Brix) colour and serum total solids (serum). Texture of the fruit (texture) does not appear to have a strong effect within the data, but was opposed to sweetness (Brix), suggesting that the firmer fruits generally had a lower Brix value. Texture, however, did not seem to group with viscosity data. This is again indicated in Figure 7-24 , where an R^2 of 0.036 has a probability of correlation of 91.7% showing there was no significant correlation between viscosity at 3.5% TS and texture of the pericarp tissue (see statistical analysis section 3.2.13.2 for test for the significance of the Pearson product-moment correlation coefficient). In conclusion, no relationship between viscosity of diluted puree and fruit texture could be established. Data in the previous chapter (chapter 6) did indicate that when there is a difference in ripeness of the fruit, texture and viscosity of the puree did correlate. However, all this data was collected on the same samples where their variation came about from the maturity of the fruit. The factors linking texture and puree viscosity when comparing different IL lines seems to be more complicated.

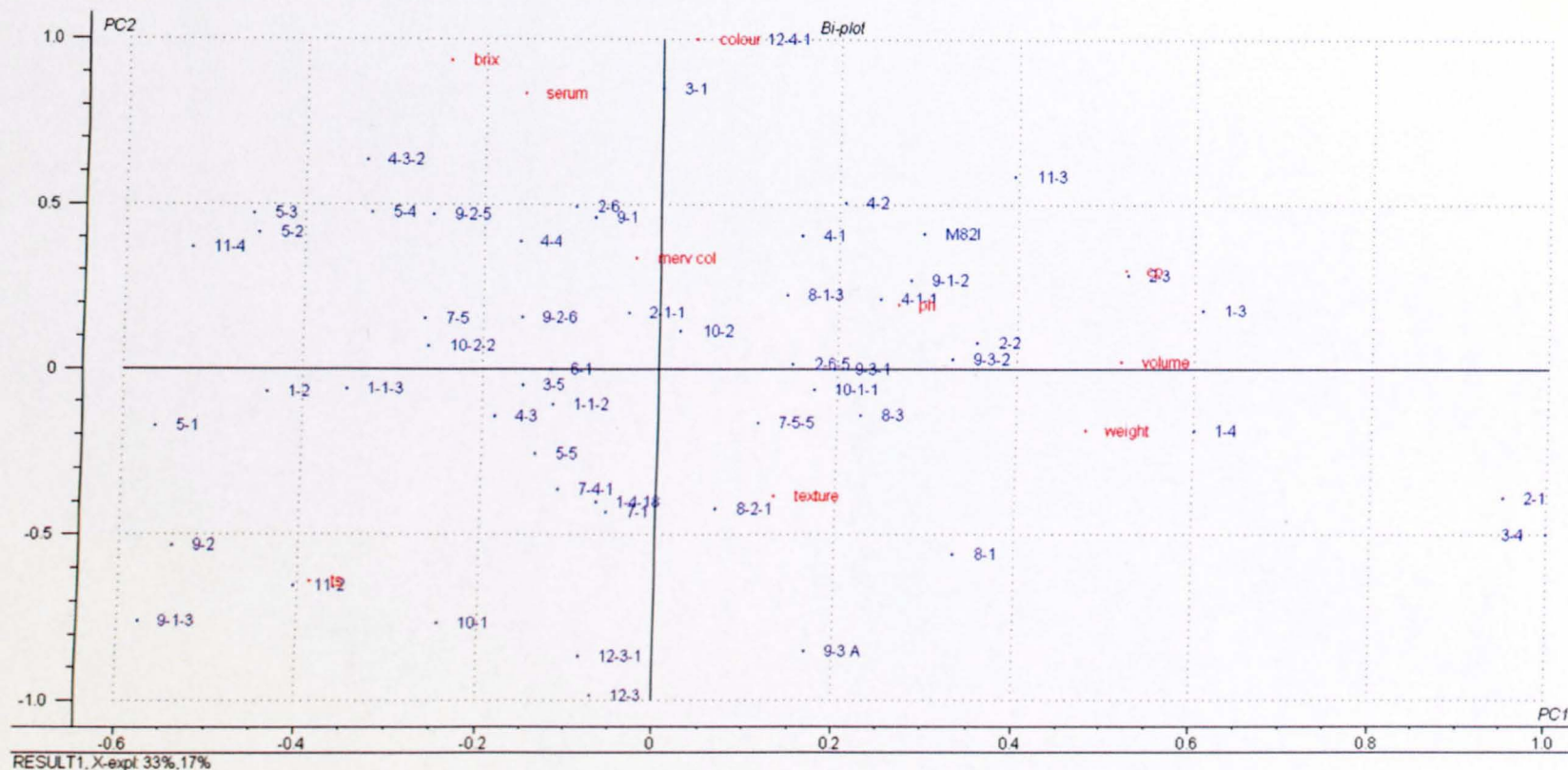


Figure 7-23 PCA bi-plot of viscosity screen for a selection of IL tomato puree samples measured for stirred viscosity at 3.5%TS harvested in 2010 compared with the texture and colour of fruits harvested at the same stage of ripeness (B+7), harvested from a crop grown in 2006. Key - ts = initial total solids, ph= pH, weight = pellet to sample weight ratio, volume= pellet to sample volume ratio, cp = stirred viscosity, colour = a* value, serum = serum total solids, Brix = Brix, merv col = a* value from 2006 data, texture = maximum load of tomato tissue, 2006

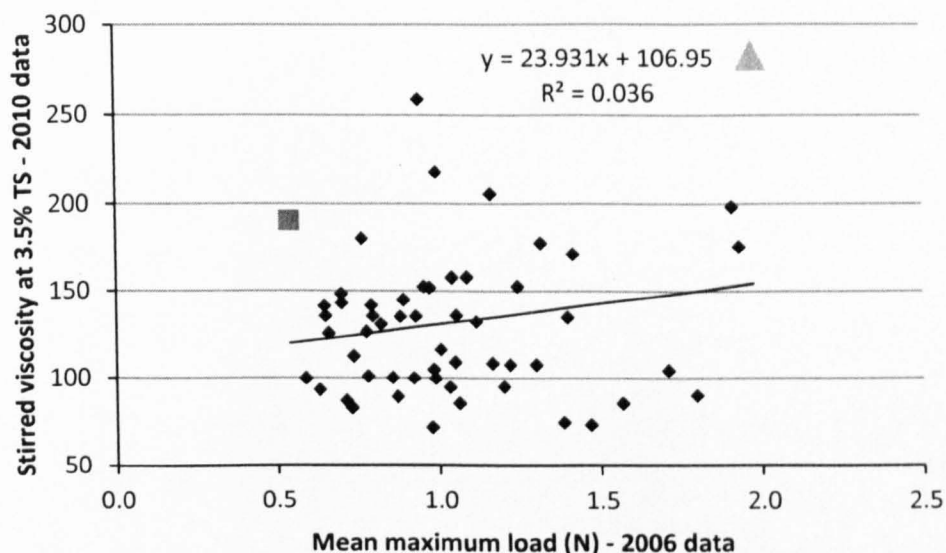


Figure 7-24 Comparison of viscosity data at 3.5% TS with previous texture data (2006)
 Viscosity measured on fruit harvested in 2010 (current study), texture, 2006 study. Square represents M82 data and triangle represents IL3-4.

7.2.7 Comparison of texture and viscosity of the control (M82) with IL3-4, an IL producing firm textured fruit

It was decided to investigate the viscosity of the introgression line IL 3-4 in more depth, in comparison to the control (M82). The introgression line 3-4 had been identified as one of the firm-fleshed varieties from the texture assessments done in previous work at the University of Nottingham by Dr Mervin Poole. The texture of the pericarp from the harvest in 2006 and the stirred viscosity values of the puree at 3.5% TS for both IL 3-4 and M82 are shown in Figure 7-24. In this figure, it shows that the IL 3-4 has a higher viscosity and a firmer fruit than the control, and had one of the highest viscosity values of the samples measured. Tomato plants were grown from these two lines, and were harvested in the winter of 2009-2010 from separate plants from the main IL viscosity screen; the colour of the fruit, the indicator of maturity, and the mean maximum load value of the inner and outer

pericarp tissue combined (ie a measure of firmness) are shown in Figure 7-25. (See methods section 3.2.3 for colour measurement method section 3.2.4 and texture methods). ANOVA results showed that a change in texture, as measured by maximum load value, was associated with the fruit colour (the texture decreased as the fruit colour changed from green through to red), and there was a statistically significant difference ($P < 0.05$) between the 3-4 and the control M82 – i.e. that even when ripeness (colour) of the fruit was taken into account, the 3-4 was a firmer line than the M82.

The breaker +7 fruits used for the full study of the introgression lines seemed to be the stage where the differences in the texture are at their greatest, as shown in Figure 7-25, and had an a^* value of skin colour of approximately 20 for the 3-4, and 24 for the M82. Samples at this stage of maturity were prepared and made into puree (see method section 3.2.6.2) and diluted to 4% total solids. The viscosity of the puree at the original total solids and after the purees had been diluted to 4% total solids was measured and the data are shown in Figure 7-26. The solids content of M82 was originally much greater than IL 3-4, but the viscosity was lower, even at this high concentration. Diluting both samples to 4% solids reduced both viscosities with the 3-4 still being higher than the control sample M82. Texture is a combination of factors including cell wall rigidity and turgor, but for the puree the cell wall residues that form particles would seem to be the key factor, and this component is present after heat treatment and mechanical breakage

(pureeing). However, when comparing the texture and puree viscosity of a larger number of ILs (see Figure 7-24), a strong relationship between quality factors of puree viscosity and texture of whole tomato fruits does not exist.

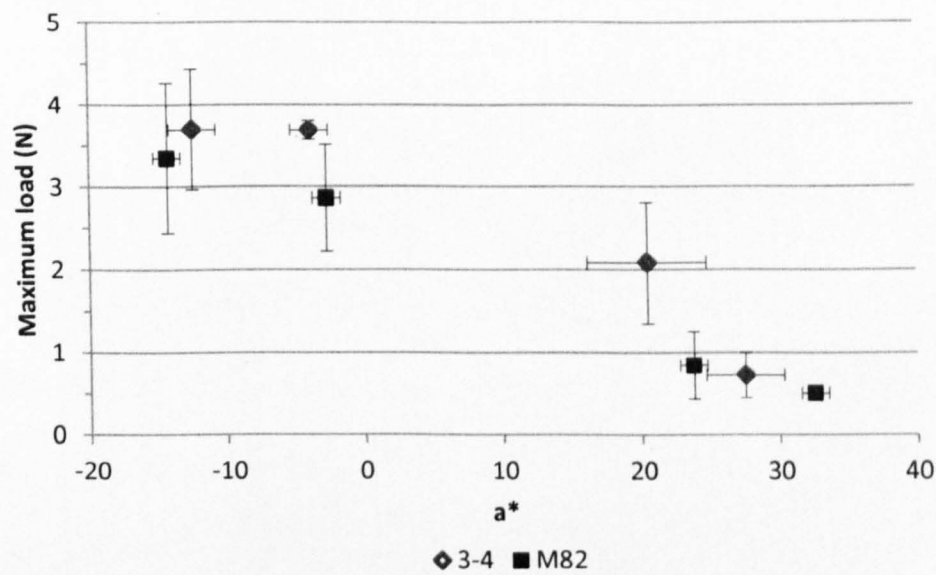


Figure 7-25 Mean maximum force of tissue (failure of tissue integrity) for firm 3-4 and control M82 – comparison of fruit firmness as a function of ripeness defined by colour. Error bars represent standard deviation of four replicate fruits.

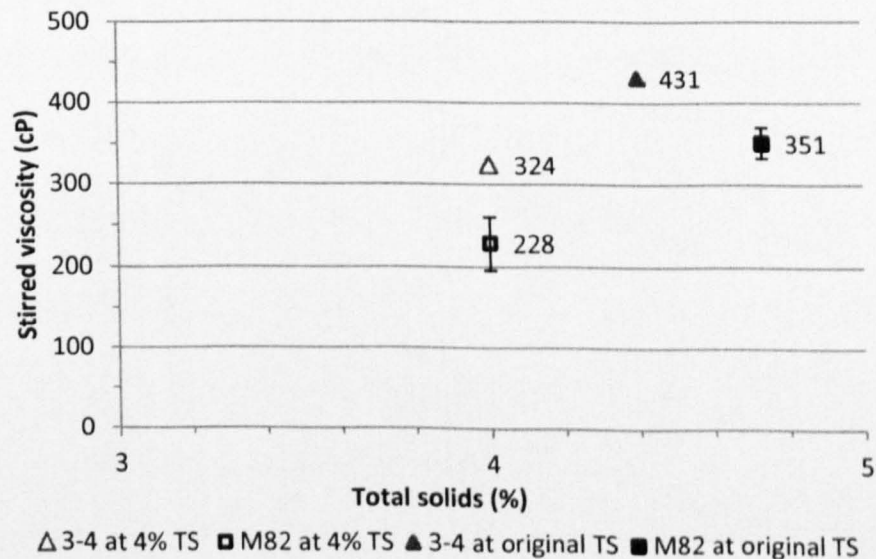


Figure 7-26 Stirred viscosity of firm 3-4 and control M821, at original total solids and at 4%. Error bars represent standard deviation of 3 replicates.

Although the IL lines were expected to show major variations in the puree quality through their genetic variation, maturity and fruit to fruit variation would seem to play a major contribution to puree viscosity.

7.3 CONCLUSIONS

Ripeness of tomatoes affects texture and puree viscosity (see chapters 4 and 6); this supports the first hypothesis (i) section 2.6. Hypothesis (iv) (see section 2.6), the main focus of this chapter, states 'at the same stage of ripeness, tomato purees prepared from tomatoes from the *S. pennellii* introgression library differ in viscosity' and suggested that viscosity could be affected by factors other than those related to ripeness. A way of effecting differences in tomato texture, without having to have fruits of different maturity is to choose those of different genetic backgrounds. The effect of genetic variation, due to the crossing of a cultivated tomato (M82) with the wild type tomato (*S. pennellii*), on tomato puree quality was therefore investigated with the expectation that not only would the relationships between texture and puree viscosity be identified, but the genetic origins for the variations in viscosity be established. Figure 2-17 shows the genome introgressions of the *S. pennellii* introgression library. Each of the 76 ILs has a small section of *S. pennellii* (wild type) genetic information on a background of the cultivated tomato (M82). Any IL showing unusual viscosity values in the experimental work described in chapter 7 therefore would identify a chromosomal segment where the genes that play a role in this attribute were

located. Any such ILs would be visually obvious in the PCA biplot shown in Figure 7-18. IL tomatoes appeared to be more or less at the same stage of ripeness when assessed on colour of the puree (a^* value) (Figure 7-1) and this should have eliminated the one major source of variation used in earlier work (Chapter 4 and 6) to choose tomatoes of different texture. Despite tomatoes being carefully chosen for ripeness (a^* colour) a major finding in the work was the variation between fruits of the same IL. The data was carefully studied, but due to the large differences in replicate values, no clear differences were observed in all the 55 ILs studied. Therefore it is very difficult to identify ILs with differences in viscosity traits.

On the data sets throughout this chapter samples have been highlighted to allow the measured parameters for these to be tracked. The control (M82) all cultivated tomato) and 5-1 (contains wild type fragment in chromosome 5) were chosen as these samples also occurred at different ends of the spectrum of results for stirred viscosity. They showed small differences in their texture measures, with M821 being less firm than 5-1 in the 2006 experimental work. When following the placement of these samples within the result sets it is clear that they alter in their proximity to one another for the different measured values. Careful investigation of the chromosome map and the order of the measured parameters show little relationship. An additional factor used to see if the genetic type influenced viscosity and associated factors was to see if any of the outliers on the correlations graphs matched

with genetic type. The outliers seems to be single values from the replicates measured and do not seem to reflect any genetic influence. Although not a strong correlation, particle size seemed to increase with stirred viscosity (as shown in Figure 7-22), indicating this may be an important factor, even when the pureed fruits are at the same stage of maturity.

It is known that the tomato with the greatest firmness is sample 3-4 and this did have the highest viscosity of the stirred puree (see Figure 7-2). However, it was reported that by Chapman et al (2012) that sample 2-3 had a firm textured fruit comparable to 3-4, but the measured viscosity for this sample although being in the top 25%, was not inordinately higher than other samples. For example it is not greater than the stirred viscosity obtained for the control M82I, which is typically reported as having poor fruit firmness.

There are inherent problems with some of the data as the measured Brix values cannot be more than the total solids, but on the whole the major variation in the data came from the variation between individual fruits. It has been reported for other quality attributes, e.g. tomato flavour, that fruit to fruit variations are very large (Linforth et al. 1994). In this current work pureeing samples and seeking to compare texture of fruits from one growing year to another will also increase error, but on the whole it must be concluded that measurement of pericarp texture does not indicate that the puree will have a certain viscosity.

Previous work demonstrated that the viscosity of the puree is dominated by the particle fraction and in this chapter it was identified that the particle fraction could well be inversely proportional to the total solids contents. It should be noted that for the majority of viscosity measures the samples were diluted to a constant solids level. This normalisation would not be possible when measuring texture. So again choice of purees with high solids or from firm tomatoes is no guarantee of a highly viscous puree.

Although ripeness affects viscosity and texture, the fruit variation (for both viscosity and texture) are so great, the confirmation that there is a direct correlation between texture and puree viscosity for fruits of the same maturity cannot be made. Hypothesis (iv) (section 2.6) would require further work before being confirmed.

CHAPTER 8: General discussion and conclusions

8 General discussion and conclusions

8.1 GENERAL DISCUSSION

There has been extensive work in the past to relate the factors in tomato fruit that affect the texture of the product, Powell et al. (2003). There is a growing interest in using fruit and vegetable puree and fibres to act as thickening agents, but the factors that would impact on the quality of these is poorly understood. Previous research has related the action of enzymes on the pectin substances and hemicelluloses with changes in the viscosity (e.g. Errington et al. 1998), but an understanding of particle creation and quality is less well documented and understood. With the extensive knowledge and genetic variations available in tomatoes, this fruit was chosen as an exemplar for the study of the particles that are a critical factor in purees.

8.1.1 Measures

Pureed tomatoes exhibit shear thinning (Figure 6-5), weak gel like behaviour (Figure 6-4) and have been measured by fundamental and empirical methods. Fundamental and empirical measurements of viscosity have also been compared (Figure 5-9).

A factor in studying the puree rather than whole fresh fruit is the processing conditions that need to be used and therefore a major challenge was to establish reproducible methods for the creation (Figure 5-12, Table 7-1), and measurement (sections 5.2.1 and 5.2.2) of tomato purees that can be used to screen materials and for more in depth study. Generally the measurement techniques and the stabilisation of the fruits were developed as reproducible methods (typically only <10% coefficient of variations for the method and creation of the puree from large batches of samples, see section 7.2.1).

8.1.2 Viscosity of puree

One of the major challenges of the work was to develop a method for the measurement of viscosity of the puree, which was a mixture of suspended particles in a solution of soluble components. Hence the puree could vary in concentration and ratio of soluble to non-soluble components. A range of viscosity techniques were used and for simple screens an empirical method using a stirred viscosity was used.

RVA as a quality method

One of the critical achievements of the work discussed in this thesis was to verify that use of the RVA, traditionally used to measure the pasting and gelling behaviour of starch and starch based products such as maize grits (Becker, Hill and Mitchell, 2001), could be used to measure the viscosity of tomato purees. The diluted samples of tomato puree have a relatively low

viscosity, when compared with gelatinised starches at concentrations typically used in the RVA. However, the RVA Super 4, the model used in the experimental work described in this thesis (see methods section 3.2.8.4), is capable of viscosity measures between 10 to 17,500 cP and proved useful for these studies at low viscosity. A limitation of the RVA is that the shear rates are variable as the paddle evokes a range of different shear regimes to the sample, but the average shear rate at 180 rpm has been calculated to be 60 s^{-1} (Lai, Steffe and Ng, 2000). This stirred viscosity has been compared with the fundamental rheological techniques at this shear rate of 60 s^{-1} and the results, although being different are not incompatible (see Table 5-1). One critical consideration for the stirred viscosity was whether the use of the paddle broke down all the particles; this did not seem to be the case (see Figure 6-18). Although the rheological data modelling the viscosity of the particles at different concentration with the Krieger-Dougherty fit established that the particles are very deformable and can closely pack, (see Figure 6-17) the RVA continuous stirring did not seem to cause more irreversible changes within the puree when compared to oscillation and shear rheology. There were some changes to the viscosity on first stirring the purees, for both the RVA (Figure 5-8) and the rheometer, but after this initial change no further irreproducible changes occurred. It is thought that the initial changes may be particle aggregation and some particle disruption, rather than all the particles disrupting.

The RVA was therefore the method of choice when screening for the tomato purees from individual tomatoes when the sample size was low. Blending of the tomatoes to create the puree required 40g and about 20g of this was used for the RVA measures. Other analyses were required for the rest of the material, including the solids content.

Use of the RVA viscosity as a measure of the introgression samples

The decision was to use this method as a screen for the introgression samples, which entailed measuring 164 samples. To avoid the complications of differences in concentration all the samples were diluted to the same concentration level of 3.5%. Generally the total solid content of red ripe tomatoes was in the region of 5% solids, with about 20% (by volume) of the material being separated from the bulk by centrifugation.

The concentration of material is expected to have an impact on viscosity, and the relationship is not expected to be linear as explained in 2.5.2. The change in the concentration of soluble solids, as these would likely to be low molecular weight, would not be expected to be as great as that coming from the particle or higher molecular weight fractions. The serum viscosity was found to be comparable to the viscosity of sucrose solutions at similar concentrations (Figure 6-6), suggesting that the water soluble material from the cell wall had a relatively minor effect on viscosity (Table 6-3). The material that precipitated by centrifugation was formed from particles and

the average size of these reduced as the tomatoes used were more ripened. The particles were generally of about 400 μ m (see Figure 6-13, and Figure 7-22) and this is equivalent to the sizes expected for tomato cells. It could therefore be expected that the agents most responsible for the viscosity were these cells and cell fragments that had been separated and broken down in the process or during the maturation process of the tomato. This finding, that the particle phase dominated viscosity, was consistent with Tanglertpaibul and Rao (1987), as discussed in section 2.1 of the literature review.

The mean particle size was largest for the mature green and decreased with ripeness and this could have been an important factor in tomato viscosity, as there was also a small but significant (probability of correlation is calculated to be >98%) correlation in puree viscosity and particle size of the purees from the introgression lines (Figure 7-22). The larger particles within the strawberry juices from genetically modified pectic lyase silenced strawberries were also associated with higher viscosity than the control strawberry juices (Sesmero et al. 2009).

The particle portion of the puree, for a red ripe tomato was about 30% (by volume) of the total (Table 6-4). In general therefore the viscosity of the puree would be affected by the fluids between the particles rather than the deformation of the disperse phase material (i.e. the particle concentration is

below close packing). The effect of particle weight as a function of the total mass of sample was measured as a function of viscosity and compared with the Krieger-Dougherty fit (Figure 6-17). This showed that the maximum packing fraction of solid spheres ($\phi_m = 0.62$) could be exceeded by purees from all levels of ripeness measured. This indicated that the particles were compressible, with the particles from more ripened fruit being more so. If it was considered that the particles were cells, it might be expected that the sucrose concentrations may be relevant to the robustness of cellular particles (osmotic pressure effects), but the same rheology of the particles occurred as long as some sucrose was present ($>1.5\%$) (Figure 6-8).

The particle volume and weight fraction, did not show a linear correlation with ripeness of the fruit (Table 6-4), suggesting there are several factors involved in the packing of the particles during centrifugation.

8.1.3 Puree characteristics and fruit maturity

Initial studies used commercially available fruits of exactly the same genetic stock, but were measured at different maturities. As the purees were generated from progressively more ripened fruits, the size of the particles decreased from over $890\mu\text{m}$ for the green tomato to $262\mu\text{m}$ for the red ripe. The processing conditions were kept constant (see methods section 3.2.6) and this size reduction probably indicates that for the green fruits the fruit flesh is

not completely broken down into singular cells, and some cell clusters are created by the maceration, while cells are broken in the more ripened fruits. Evidence for differences in particle size and structure were shown by particle size analysis, Figure 6-13, and microscopy, Figure 6-12. During the maturation of fruits it is the middle lamella between the cell walls that declines and hence the cell:cell adhesion would be decreased (Brummell and Harpster 2001). Not only does fruit ripeness impact on particle size, it also changes the solidity of the particles, with the puree from green fruits being more robust. This robustness would seem to originate from the cell component per se rather than turgor pressures as the fluid in the supernatant has little impact on the rheology of the particles. It is not surprising that the cellular wall mechanisms are no longer relevant in the puree as all the samples were heated to over 90°C to stop enzyme activity. Breakdown of the pectic materials between the cells might have rendered them soluble and it could be expected that the soluble macromolecules would have high hydrodynamic volumes and therefore increase the viscosity of the continuous phase. The viscosity of the supernatant did not increase substantially on ripening (Figure 6-6) and the critical factor was the particles.

Viscosity of purees did alter, even when tomatoes were of approximately the same ripeness, as measured by the redness of the fruit (Figure 7-9, and Figure 7-26).

It has been established that tomatoes of different genetic origins have different textures (Chapman et al 2012). To investigate purees from a range of tomatoes the *Solanum pennellii* tomato introgression lines (ILs) were grown and harvested from the glass houses at the University of Nottingham. A screening process was developed to compare various parameters of puree created from individual tomato purees. This was carried out on 55 of the ILs. The tomatoes were picked so that they were of similar ripeness (breaker plus 7 days). Parameters measured appeared to either affect the quality of the puree (viscosity, total solids, particle volume and weight) or the eating quality of the fruit (Brix, a measure of sweetness, serum solids or colour) Figure 7-18). There was a strong correlation between the viscosity of purees at 3.5% TS and the particle (pellet) to sample weight ratio (Figure 7-16), but these were negatively correlated with the initial solids content of the puree Figure 7-17) before dilution. Therefore samples with high initial solids content may not contain high levels of particles. The initial work carried out using fruits of different ages showed very little differences in the solids content of the puree (Table 6-1). This variation in puree concentration may therefore be genetically controlled. However despite in depth investigation of the measured parameters carried out on the purees, it was not possible to link any factors to the known genetic variations. Perhaps a major reason for the difficulty in linking the genetic variation with the measured parameters was the fruit to fruit variations.

In this study texture of the IL line samples used to create the purees was not measured. The texture measures came from work carried out on the same lines but grown four years previously. Using average data for puree viscosity (at 3.5% TS) and average maximum load data there was no correlation (probability of occurring by chance was greater than 10%) (Figure 7-24). It therefore can be concluded that the texture of the fruit and the puree viscosity both decrease as the fruit ripens, but at any one stage of maturity a firm fruit may not give rise to a high viscous puree.

8.1.4 Final conclusions

The puree system is complex and depends on many different factors. However a dominant feature appears to be that in the tomato system the major component of the particle phase originates as the tomato cell. In less ripe fruits it appears that clusters of cells are stable in the puree and these have large hydrodynamic volumes, but are not easily deformable therefore small quantities of these can dramatically increase the viscosity of the matrix. When at the same stage of ripeness (breaker+7 days), different IL lines of tomatoes from the *S. pennellii* library have different solids content and the ratio of particles to serum decreases; a high initial total solids correlates with a low pellet ratio on dilution to 3.5% TS . If fruit fibre or particle material is to be used as a thickener then use of cellular material from green tomatoes could be recommended. However the use of green fruits may impact on the organoleptic properties.

Choice of fruit to yield high viscosity puree

- The work reported in this thesis shows that tomatoes can yield a puree with a high viscosity and will contain a fibrous element that is not affected by the sugar content. This could make these types of puree useful thickeners with low calorific values and increase digestible fibre. This will contribute towards the recommended nutrient intake of 18g per day of NSP (dietary fibre) (The Department of Health, 1991).
- To maximise the viscosity the critical factors is the amount of insoluble solids. It is known that these change during ripening, but other factors could also be important. Although the work on the introgression lines demonstrated the wide variation that occurs fruit to fruit, the general choice of high viscosity purees is still likely to be related to low cell wall breakdown factors. Although there could well be a difference between cell-cell adhesion that dominates texture of whole fruit and the actual cell wall integrity which is the important factor for the puree rheology.
- For maturity the better choice would likely be the mature green tomatoes, which have been concluded to have robust particles. Although some recovery of the particles from over ripe tomatoes could be possible, a great deal of material would be necessary to give comparable viscosity to less ripe fruits.

- Eating of large quantities of green tomatoes is not advisable due to the presence of tomatine. However, this alkaloid could be removed leaving the particulate fibrous material and this would be perfectly acceptable as a thickener for soups sauces and other foods requiring thickening with natural materials.

Chapter 9: Future work

9 Future work

9.1 THE EFFECT OF RIPENESS ON THE RHEOLOGY OF PUREES WITH THE SAME PARTICLE SIZES

Investigation into the effect of particle size would be interesting and would help ascertain the contribution of the particle fraction of puree samples made with fruits at several different stages of ripeness, without the particle size being a significant factor. This simple concept would need careful experimental execution, as ripeness has been shown to be associated with differences in the mean particle size and particle size distributions of the particles in puree. A possible approach would be to use a preparation method for hot break puree for tomatoes as described in this thesis and use screens or sieves to break up the larger particles during the preparation process, as is often done in commercial tomato paste production (see section 2.1). Another method could be to use a wet sieving technique where puree is passed through a series of sieves placed on top of one another and the puree is allowed to pass through the sieves, thus obtaining several samples each with particles of a narrow and defined size range. The rheology of these subsamples could be measured, taking into consideration other parameters such as total solids, Brix and pH, particle phase (pellet) volume.

9.2 THE EFFECT OF INTROGRESSION LINE ON RHEOLOGY OF PUREE

Since fruit-to-fruit variation was found to cause difficulty in identifying the effect of genetic variation on tomato puree viscosity, a few of the IL lines from the *S. pennellii* range could be used for a further study. In this more detailed study, more replicates of individual fruits could be measured, or bulk samples of more than one fruit could be studied (with replicates) to try and overcome the fruit to fruit variation. The following lines would represent ILs with high and low puree viscosities (see Figure 7-2) and would make a suitable starting point for this experimental work;

ILs with highest viscosity,

3-4, 2-4, 1-3, 11-3, 1-4, 2-3

ILs with the lowest viscosity

5-1, 9-1-3, 11-2, 9-2-5, 1-1-3.

It is suggested that the control sample (M82) and some samples with intermediate viscosity could also be measured.

On these samples it is suggested that the more replication could be made, and the viscosity measurements taken at the non-diluted total solids content, as well as at the same solids level. The robustness of the particles (Krieger-Dougherty plot) could be studied and the types of and quantities of hydrocolloids in the cell wall assessed.

If any of the above lines demonstrated statistically significant very high or very low viscosities, work could continue to the next stage to pinpoint the putative genes responsible for viscosity;

The overlapping nature of the introgression lines makes bins. These are important in that they allow a greater degree of precision to locating the area of chromosome important in the phenotype of interest. For example, in the diagram in Figure 2-17, if IL 1-4 and 1-3 shows the desired phenotype, but 1-2 and 1-4-18 does not, then the area of the chromosome containing the genotype responsible must lie on the area overlapping 1-4 and 1-3.

Refinement of the process would follow next, where (for example) 1-4 (the IL of interest) is pollinated with the M82 again to generate sub lines. The markers are defined again to identify the area on the chromosome and the tomatoes grown from those lines are screened again for the desired phenotype. This is done to determine to a greater precision which part of the chromosome contains the gene of interest. This further screening process can be repeated until the smallest possible piece of wild type remains, which contains the gene of interest.

9.3 EFFECT OF CELL WALL MATERIAL COMPOSITION ON TOMATO RHEOLOGY

An area not investigated in any great depth in this study was the effect of the individual polysaccharides of the cell wall on rheology of the puree. This could be done on tomatoes at different stages of ripeness, or with known genetic variation (such as PG antisense tomatoes), or ILs such as those identified in section 9.2. For this work to be carried out it is suggested that cell wall material could be extracted from the tomatoes after they had been prepared into puree. The quantity of the cell wall material could be compared, and a sequential extraction of the cell wall polysaccharides could be made to separate the pectins hemicelluloses and cellulose. Parameters such as the molecular weight, molecular weight distribution, the intrinsic viscosity and visco elastic properties of these hydrocolloid chains from these extracts could be measured (e.g. size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS)). Some of these methods have been used in work by Harding et al (1991). The measurements made on cell wall extracts could be compared with the rheology and material properties of the puree. This would begin correlation of the hydrocolloids and the puree with ripeness and or genetics.

9.4 POSSIBLE FUTURE INITIATIVES

9.4.1 Other vegetable matter

The reason for looking at tomato puree was to get a generic idea of the structures that dominated fruit behaviour after maceration. The requirement

within foods of thickeners that are considered natural and of high dietary quality continues to increase. The concept that it is the cellular components that are important could now be tested by using other fruits and vegetable materials. Often green waste is particularly robust in terms of cell structure. Waste fruit or vegetables could be macerated and the particulate investigated using the methodologies developed in this thesis to see if this would provide a fibrous thickener suitable for inclusion in other foods.

9.4.2 Fruit to Fruit variation

One key feature of the work was the great variation in fruit to fruit variation in the IL work. This variation may have been due to dilution of the purees to the same solids concentration, but a much more in depth study of cellular structures in fruits of similar maturities could be helpful in establishing fruit harvesting patterns. It has been shown that other quality attributes (e.g. flavour) differ fruit to fruit, but an understanding of why is still not generally known. The IL lines developed and the methods created for purees would make further detailed studies possible.

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Appendix 1

Appendix 1 is a copy of the co-authored book chapter of Gums and stabilisers for the Food Industry 16, published in 2012 by Woodhead Publishing Ltd.