Effect of cultivar and growth region on the mechanical and biochemical properties of canned baked beans

Jin Chu

Submitted in accordance with the requirements for the degree of PhD (Doctor of Philosophy)

The University of Leeds
Food Science and Nutrition

December, 2014

The candidate confirms that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others

2014

The University of Leeds

Jin Chu

Abstract

Canned baked beans are the most widely consumed legume product in UK. The canning quality of the beans will significantly influence consumer acceptability and the commercial sales. In this project, the mechanical, microstructure and biochemical properties of several cultivars of navy beans which were grown in different regions of North America were investigated in order to identify factors that could help the industry to predict canning quality.

A texture analyser (TA.XT plus) was used to measure the mechanical properties of the beans, either as single or a batch of beans. Beans from cultivar 2 and region C had significant firmer texture, and region affected firmness more than cultivar. Beans canning in tomato sauce softened by 98% compared to blanched beans, and achieved 50% firmer texture than those canned in brine or water. The mechanical properties may have been influenced by the structural properties. Cytochemical and immunofluorescence microscopy was used to visualise the microstructure of the beans' skin and cotyledon, with a focus on the localisation of cell wall polysaccharides. Cell adhesion in the cotyledon was mediated by un-methyl esterified HG localised in the middle lamella, which was not completely lost upon canning, most likely due to osmotic pressure from the sauce. However the differences between cultivar and region could not be quantified using the microscopical techniques. Exchange of polysaccharides was observed between the bean and the sauce. Analytical biochemical methods were used to analyse the composition of the beans. Canned baked beans were rich in dietary fibre (10.8%-16.4%) which was composed of cell wall polysaccharides and undigested starch. Firmer beans were found to contain more dietary fibre (r=0.85), most particularly cell wall neutral sugar (r=0.82) composed of arabinose, galactose, xylose and mannose. Therefore, cell wall structure and composition are affected by cultivar, growth region and processing conditions, and are very important in determining the textural properties of canned baked beans. However, of all the physical, mechanical and biochemical measures studied in this thesis, only one (drained weight) was highly correlated with the other measures, and significantly affected by cultivar and region. Therefore, drained weight may be the best predictor of textural quality.

Table of Contents

List of Tables

List of Figures

Chapter 1
Introduction

1.1 Navy beans

Legume seeds are widely consumed by people because they offer great nutritional benefit. They are a good source of protein (20% - 38%) and complex carbohydrates (50% - 60%) (Rehman and Shah, 2005). Besides, they are also rich in fibre, minerals, vitamins, polyunsaturated lipids and potassium (Rehman and Shah, 2005, Zhao and Chang, 2008). At the same time, they are low amount of sodium, have no cholesterol and contain traces of saturated fat (Zhao and Chang, 2008). The regular consumption is observed in both Mediterranean and Asian style diets. There was evident showed beans consumption associated with reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and disease of the digestive tract (Anderson et al., 1984, Geil and Anderson, 1994, De-Mejia et al., 1999, Bazzano et al., 2001). It also indicated that a diet rich in legumes other than soy decreases total and LDL cholesterol (Bazzano et al., 2011). Recent studies also found that regular consumption of canned navy beans reduces metabolic risk factor in overweight and obesity (Mollard et al., 2012, Luhovyy et al., 2015, Rebello et al., 2014).

Navy bean is a type of common bean (phaseolus vulgaris) with round white seed in the weight range from 180 to 240 mg/seed. It is one of the most important widely consumed legumes, especially in the United Kingdom and the United States of America, where is consumed canned in tomato sauce. The majority of the navy beans consumed in the UK are imported from North America, mostly from Manitoba and Ontario. Figure 1.1 shows the structure of navy bean seed. Navy bean is a dicotyledonous plant, therefore its seed contains two cotyledons surrounded by a skin (seed coat). Micropyle gives rise to and contains the female reproductive cells. Hilum is a scar left on the seed coat by the former attachment to the ovary wall, is called the ‘eye’ of the seed. Radicle develops into the primary root of the plant after germination. Hypocotyl (meaning ‘below seed leaf’) is the stem of germinating seedling which is above the radicle. Epicotyl is the embryonic shoot above cotyledon, and eventually develops into leaves of plant. Plumule is the young shoot of a plant embryo. In food science, the cotyledon is the most significant part of the seed that related to sensory quality, and the skin provide the first protection for the

seed. Therefore, the properties of the skin and cotyledon of the navy bean will be studied in this project.

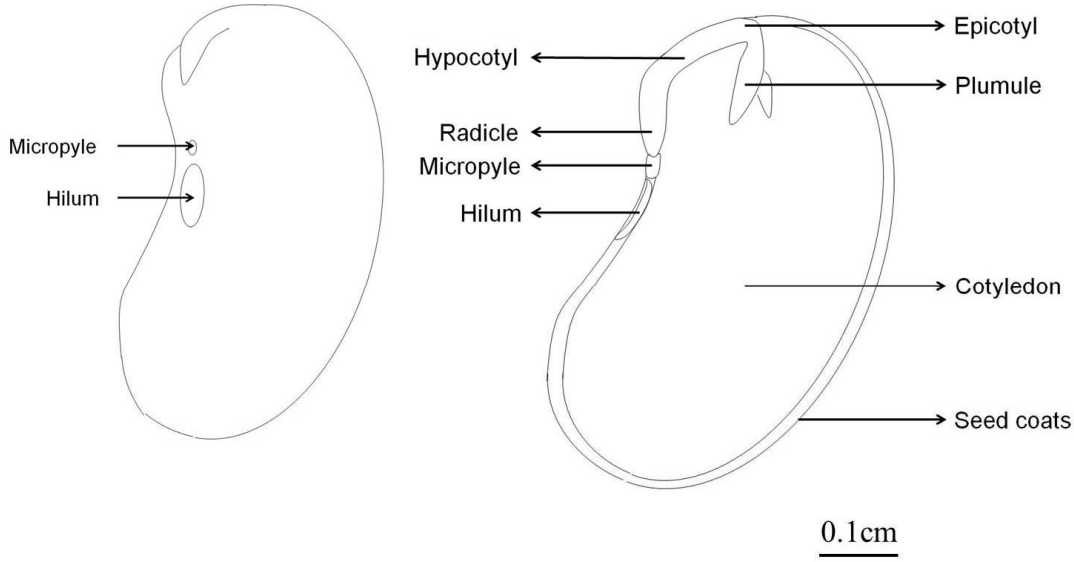

Figure 1.1 Schematic diagrams of navy beans structure.

Canned baked beans have been shown to offer great nutrition benefit as well. Their regular consumption has been shown to lower total cholesterol and low density lipoprotein cholesterol levels in plasma and reduce cholesterol deposition in liver (Shutlera; et al., 1989, Winham and Hutchins, 2007, Costa et al., 1993, Bazzano et al., 2011). Furthermore, saponins in the navy beans were also found to inhibit cancer cell growth and exhibit antibacterial and anti-fungal activity properties (Shi, Xue et al. 2009). Navy beans also contained the highest content of the phenolic acids ferulic acid and p–coumaric acid among the common bean varieties (Luthria and Pastor-Corrales, 2006).

Canned baked beans earned British population's widely adoration because of its cheap price, as well as the abundant benefit mentioned above. Canned baked beans are the most widely consumed legume product in the UK, with around 50% of the population consuming baked beans at least once a week. The average consumption is around 300 grams per week, higher than other cooked vegetable source including peas and carrots (Henderson et al., 2002). As the biggest baked beans supplier in the world, H. J. Heinz Company produced 1.5 million cans of baked beans per day. Hence, canned baked beans can be considered a staple product in the UK diet. It is therefore very important to understand the texture quality of the canned baked

beans, in order to optimize quality for both customer satisfaction and commercial benefit.

Table 1.1 lists the studies that have investigated the texture of canned baked beans. The table shows the measurement and main aims for these studies. Most of them studied the mechanical properties of canned baked beans from different cultivars and regions, compared different texture analysis methods or canning procedures to evaluate the canning quality. Very few studies work on the chemical composition of canned beans. Wang and Chang (1988) and Lu and Chang (1996) studied the yield of soluble pectin of canned beans, and correlated with mechanical properties of canned beans. Or investigate the chemical composition including protein, Ca, K and correlated with canning quality of beans (De Lange and Labuschagne, 2001). None of these studies investigated the structural or various chemical factors (carbohydrates including starch and fiber) that could determine the texture quality of canned beans. With advances in genomics and plant breeding, combined with advances in analytical techniques, there has been an increase in the demand for plant cultivars with particular agronomical or food quality attributes. Therefore, the effect of cultivar and growth region on various selected structural and chemical factors that affect bean textural properties were studied in this thesis.

Table 1.1 Comparison of studies on canned baked beans

Reference	Cultivars	Regions	Instrument	Measurement	Main aims
(Voisey and Larmond, 1971)	4	Ontario	Instron universal testing machine	Kramer shear ; Back extrusion; Wire extrusion; Plate extrusion; Pea Tenderometer	Comparison of several methods of measurement
(van der Merwe et al., 2006)	4	9 in South Africa	FTC texture press	Load cell	Comparison of canning quality of laboratory and industrial techniques.
(De Lange and Labuschagne, 2001)	6	11 in South Africa	Texture Press	Multiblade shear compression cell	Correlation between protein, Ca, K and canning quality.
(Anzaldua-Morales and Brennan, 1982)	6	N/A	Instron universal testing machine	Puncture; Compression; Kramer shear; Extrusion	Correlations between mechanical properties and sensory evaluation
(Lu and Chang, 1996)	11	2 in North Dakota	Instron universal testing machine	Kramer shear	Correlations between soluble pectin and canning quality
(Warsame, 2014)	29	N/A	Texture Analysor	Heavy duty platform	Comparison of different cultivars canning quality
(Walters et al., 1997)	3	2 in Michigan	Instron universal testing machine	Kramer shear	Canning quality heritability (DNA) estimate

(Wang et al., 1988)	3	Dakota	Instron universal testing machine	Back extrusion	Canning processing evaluation
(Wang and Chang, 1988)	1	Dakota	Instron universal testing machine	Extrusion	Canning method evaluation
(Balasubramanian et al., 2000)	1	North America	Food Technology Corporation	Compression; Kramer shear	Laboratory canning protocol evaluation

N/A not available

1.2 Texture of food

‘Texture is the primary response of the tactile senses to physical stimuli that result from contact between some part of the body and the food. The texture properties of food are sensed primarily by the feeling of touch, are related to the deformation, disintegration, and flow of the food under a force. Texture properties are a group of physical characteristics that arise from the structural elements of the food, and can be measured objectively by functions of mass, time, and distance’ (Bourne, 2002). Food texture is very important because it plays an important role to the overall food quality. For example, for the meat or celery, the food texture is the dominate quality characteristic; for the fruits or cereal-based food, the food texture makes a significant but not a dominant contribution to the overall quality. As people obtain great enjoyment when they eat the food, understanding the texture acceptability factors of food are extremely important. Achieving the desired food texture quality has important economic considerations for the food industry. Achieving the desired texture quality for the baked beans is the one of food industry’s top R&D priorities. This is due to the fact that most costumers complaints relate to inadequate texture attributes. For examples, hard beans with a thin sauce, or mushy beans with a thick sauce. Heinz has also observed large variations in quality between its raw bean suppliers and also year on year from the same supplier. Therefore, there is a need to understand the relationship between cultivar and agricultural conditions on bean textural quality.

1.2.1 Effect of processing on food texture

As most native food cannot be consumed raw, food processing is usually applied generally directed to weaken the structure of the food, to make it easier to masticate, swallow and digest. For example, raw navy beans are too hard for humans to chew. Raw beans have poor digestibility due to their compact structure and the presence of protease inhibitors. Food processing of beans include blanching and canning to achieve a digestible and desired texture quality. Blanching relies on a brief exposure to a heat transfer medium at a relatively high temperatures, it aims to inactive the enzymatic reactions which leading to changes in colour, flavour or texture of the food, but does not cause dramatic changes to food structure. Pre-blanching was found to be of greatest importance for green beans to be canned and sterilized in tins (Kaack, 1994). Vegetables such as green beans, potatoes and carrots which are

preheated at moderate temperatures (50°C – 90°C) for an appropriate period of time before future processing remained significantly firmer than conventionally processed vegetables (Lin and Schyvens, 1995, Andersson et al., 1994, Sajjaanantakul et al., 1989). This effect is consistent with general idea that blanching affects enzyme activities (PME) in the cell wall (Stolle-Smits et al., 2000, Bartolome and Hoff, 1972). Pectin methylesterase (PME) was active above 50°C and react with pectin, increase the amount of carboxyl group which allowed calcium to form the metal bridge, and increased the firmness of the beans. On the other hand, preheating effect can be attributed to demethylation of pectin by endogenous PME during the preheating period, reduced the β-elimination reaction, which was believed to most important softening process during sterilization (Stolle-Smits et al., 2000). These reactions prevented the destruction of bean structure. Balasubramanian et al. (2000) claimed that blanching temperature did not have a significant effect on the texture of the beans or the canning quality traits (including hydration coefficient, washed drained weight and percent washed drained weight), meanwhile, hydration above 80°C for a short time (3-4h) required approximately the same energy as hydration at room temperature for 14-16h, but the overall process duration can be reduced significantly (14-16h to 3-4h). Hence blanching at 70°C - 85°C for 20 - 25min was widely used by industry, same process was used in this project in the laboratory to simulate the industry processing.

Canning is heating food at high temperatures under pressure, usually within a metal can. The cans were sterilized at 110°C – 128°C depends on different manufactures. It aims to inactivate microorganisms but also has quite substantial changes to food structure and food chemical properties. The texture changes of products that are acidic such as tomatoes are not qualitatively different from those observed in ordinary cooking procedures. However, the texture of products that are less acidic such as beans and potatoes are likely to be more pronounced due to changes to depolymerisation of pectins (Kramer and Szczesniak, 1973). The heat treatment applied to the food causes the destruction of the selective permeability of the cell membranes and the internal pressure of cells (and turgor) is permanently reduced. Some cell distension may still be present because of hydrophilic but non-diffusible substances in the cell such as starch (Kramer and Szczesniak, 1973). Kramer and Szczesniak also indicated that tissues immersed in syrup during canning not only exhibit an uptake of carbohydrate by diffusion but also appreciable adsorption on the

cell walls. The net effect of these transfers of solutions is an increased firmness of food.

1.2.2 The factors determining food texture

Canning results in dramatic changes to the texture of beans, with a dramatic softening being observed even though the beans retain their shapes. The factors that determine the texture can be arranged into three dimensional levels, as shown in Figure 1.2. Texture is related at the macroscopic level to physical and mechanical properties such as water imbibition of the seed and firmness of the skin and cotyledon (Anzaldua-Morales and Brennan, 1982). In canned baked beans, sauce viscosity also significantly affects the overall acceptance of the product quality. The mechanical properties are determined by microstructural properties which include cell size and shape, cell adhesion/cohesion, and cell wall architecture. For example, cooked Lima beans softening was found to be contributed largely by loosening of the intercellular matrix of the middle lamella to allow separation of cells without rupture of cell walls, resulting in a mealy or grainy, yet soft texture (Rockland and Jones, 1974). Microstructure properties, such as cell-to-cell adhesion, are important determinants of the textural properties of fruit and vegetables because they affect the way food breaks when people mastication (Waldron et al., 1997). Microstructure properties are finally determined at the molecular level by chemical properties which include the composition of the cell wall components, and their interactions. Cell wall components, such as pectins (including HG and RGI), have been shown to contribute to the cell adhesion (Caffall and Mohnen, 2009, Mohnen, 2008). Therefore, cell wall structure and cell wall composition are considered as very important determinants of texture. All the factors shown in Figure 1.2 will be studied and discussed in this project.

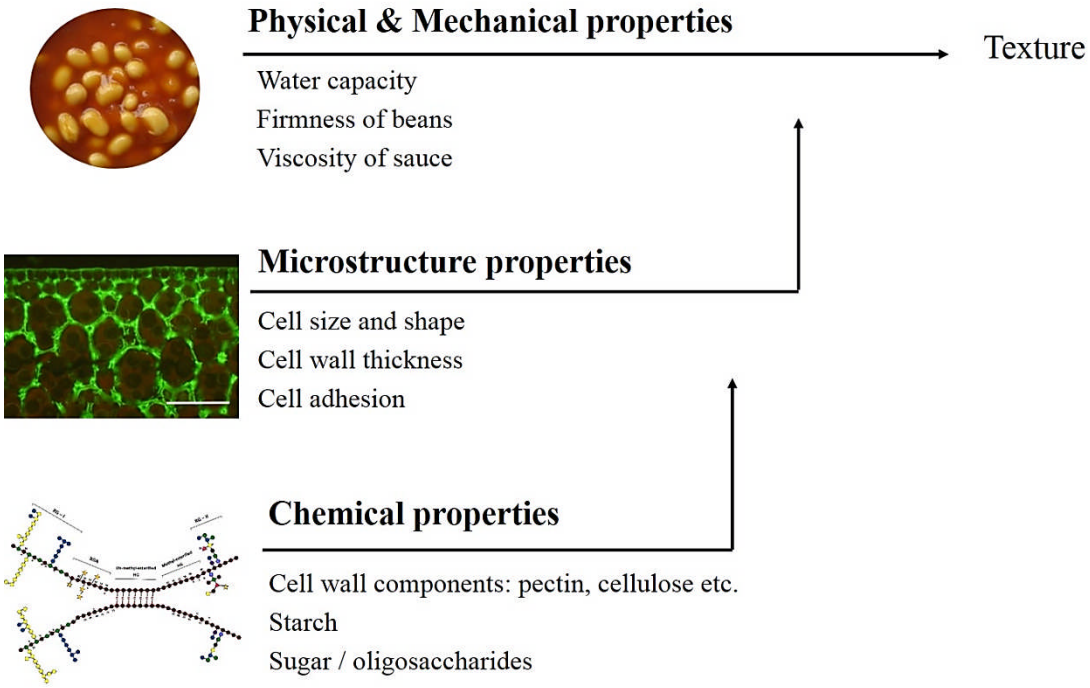

Figure 1.2 Schematic representation of the determinants contribute to the textural properties of beans.

1.3 Measurement of the mechanical properties

A wide variety of methods have been used to measure the texture properties, such as fundamental tests (measure well-defined physical or rheological properties, e.g. viscosity), empirical tests (measure poorly-defined physical or rheological properties, e.g. sensory test) and imitative tests (imitate the condition of chewing process) (Rosenthal, 1999) . In this project, fundamental tests were used to measure the texture quality of canned beans using instrumental methods. The most widely used instruments used to measure the firmness of food include the Instron Universal testing Instrument, a similar instrument called the Universal Texture Analyser (Wang et al., 1988, Lu and Chang, 1996), Kramer shear press instrument (Walters et al., 1997, Lu and Chang, 1996, Balasubramanian et al., 2000, Anzaldua-Morales and Brennan, 1982) and Ottawa system (Voisey, 1971, Voisey and Larmond, 1971). In this thesis, the Universal Texture Analyser (TA.XT plus) with different probes (including 2mm diameter cylinder, 20mm diameter cylinder, Miniature Kramer shear cell and Ottawa cell) are used to measure the textural attributes of navy beans. A number of food specific methods exist within the instrument. However, new methods were created for analysis of the beans, either individually or as a batch.

In this project, hardness is described as the perceived peak force required to break the sample. Toughness is described as the total work needed to break the sample. Firmness is described as the joint name for hardness and toughness. As shown in Figure 1.3.

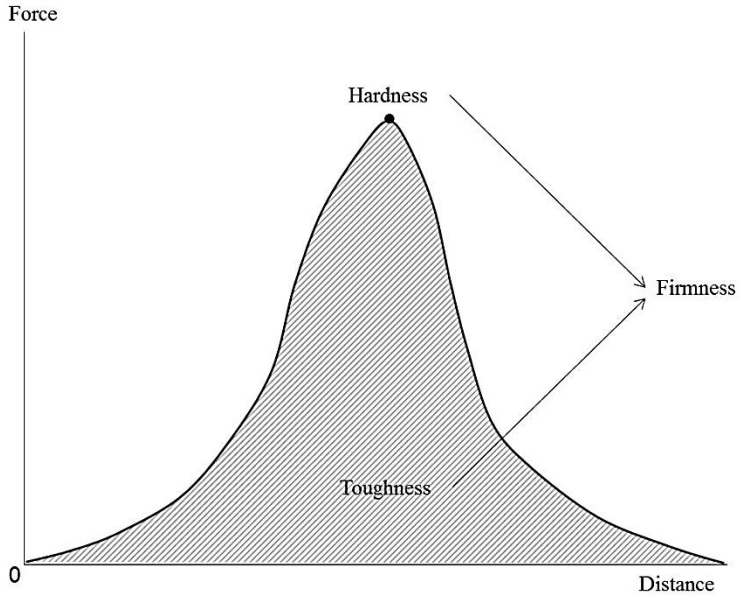

Figure 1.3 Schematic figure shows the definition of hardness, toughness and firmness of beans in this project

1.3.1 Puncture test

The puncture test measures the force required to push a probe into a sample of food. When a puncture test is undertaken using the TA.XT, a force versus distance or force versus time curve was drawn, both of which are dependent on the speed on puncture. Previous study indicated that speed did influence the results but not to the extent that variations between different samples are altered (Anzaldua-Morales and Brennan, 1982). A puncture test can produce three basic types of curves shown in Figure 1.4. In curves A, B and C, there is an initial rapid rise under the force for a short distance. During this step, the sample is deforming under the load but no fracturing to the tissue has occurred. The probe begins to puncture the food and the deformation step abruptly ends. It represented as a sudden change in slope called ‘yield point’. This yield point is of great interest because this point marks out the force that cause the irreversible fracturing or crushing of the tissue (Mohsenin et al., 1963). After yield point, a third phase of begins associated with further deformation and simultaneous breaking. Type A force continues to increase after the yield point

and there is a continuous positive slope of the curve, Type B force is approximately constant after the yield point and there is a continuous approximately flat slope of the curve. Type C force decrease after the yield point and there is a continuous negative slope of the curve. The sensory and physical meaning of the difference between types A, B, C is presently not well understood but cases of food with type A profiles of increasing positive slope are in a very limited number of cases (Bourne, 2002), and are associated with multiple layer structures, for example starch pastes and whipped toppings are essentially type A curve but without a sharply delineated yield point. There are cases for type B curve which where the force continues after break compresses the structure, for example, freshly harvest apples. Finally, types C results in a breakdown on the structure which allows the probe to penetrate easily into the food, for example, baked beans in this project.

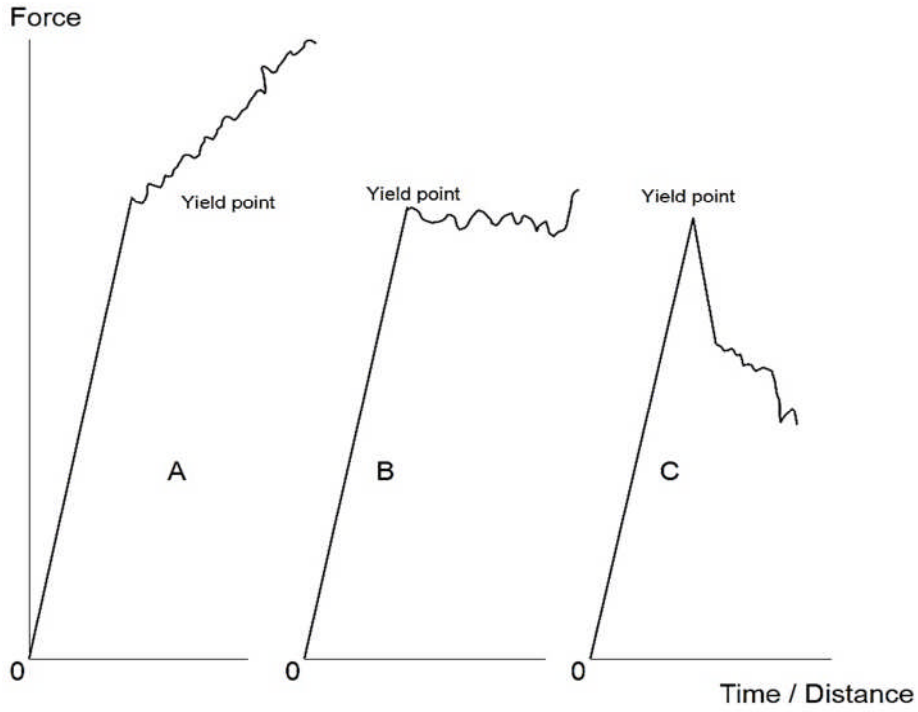

Figure 1.4 Schematic diagram of typical force *vs.* distance or force *vs.* time curves obtained from a puncture test. (Adapted from (Bourne, 2002))

Figure 1.5 shows the diagram that illustrates the procedure of a puncture test for a single bean using a 2mm diameter cylinder probe. For assessment of hardness of the skin, the sample should at least 3 times longer than the probe diameter as shown in the Figure 1.5.The probe puncture the approximately centre of the bean, and penetrate through the skin to obtain the yield point as the hardness of the skin.

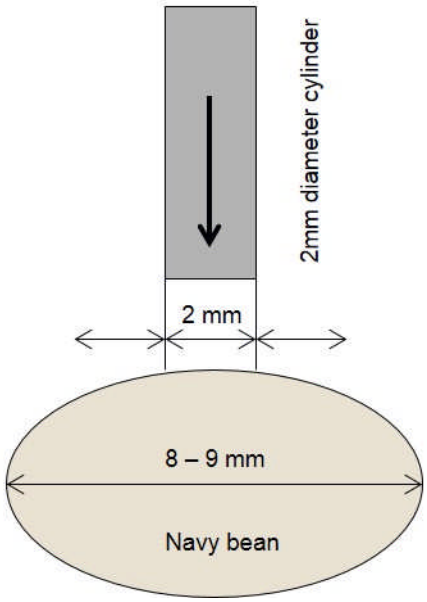

Figure 1.5 Schematic diagram of puncture test for a single navy bean using 2mm diameter cylinder.

1.3.2 Compression test

The compression test is applying a compressive force to a sample of food to a pre-determined distance. The food is disrupted by the compression. The total work required to accomplish the compression is measured and used to determine the firmness of the food. Figure 1.6 shows a diagram that illustrates the procedure of a compression test for a single half bean using a 20mm diameter cylinder probe. For assessment of individual beans, the sample should be smaller than the probe as shown in Figure 1.6. Because the two cotyledons were very easy to split under the pressure and interrupted the results. Hence, only one cotyledon was analysed (half bean) to increase consistency. The probe compressed and crushed the cotyledon to obtain the total work as the toughness of half cotyledon.

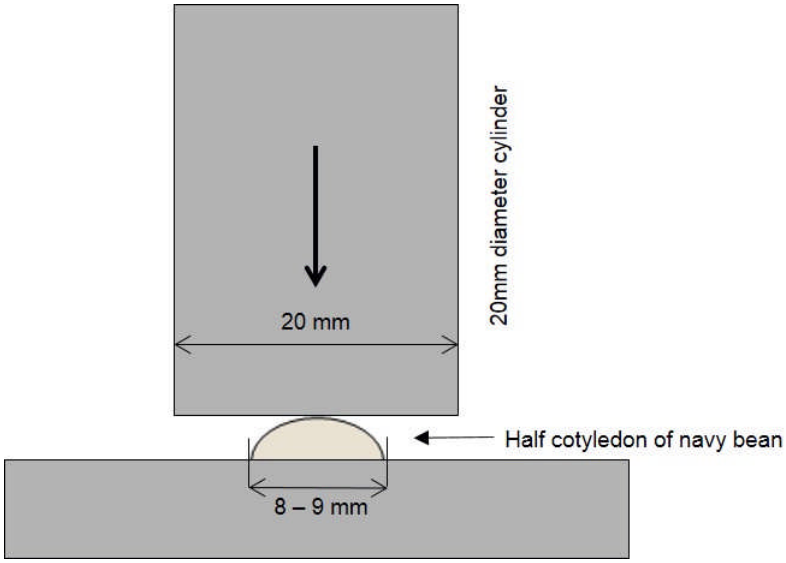

Figure 1.6 Schematic diagram of compression test for a single half cotyledon of navy bean using 20mm diameter cylinder.

1.3.3 Extrusion test

The extrusion test is applying a force to a food sample until it deforms and passes through an outlet that may be in the form of some slots or holes in the test cell. The food is compressed until the structure is disrupted and it extrudes through these outlet. The maximum force required or total work required to accomplish the extrusion is measured and used as an index of the food texture quality (Bourne, 2002). The extrusion test consists of 'back extrusion test' and 'forward extrusion test'. The test used in this project was 'forward extrusion test' because the food moves in the same direction as the plunger. The opposite occurs for back extrusion, used to measure, for example, the stickiness of foods.

1.3.3.1 Kramer shear press measurement

The standard cell of the Kramer shear press applies a combination of extrusion and shearing but little compression force. A constant sample weight should be set when testing food because for most foodstuffs, the force required to deform a sample is not constant but decreases as the sample weight increases (Bourne, 2002). In this project, 10g of beans samples were used for each test.

Figure 1.7 shows the diagram to illustrate the procedure of a Kramer shear press measurement for a whole batch of navy bean (10g) using a miniature Kramer shear cell. Part of the food is extruded forward through the slits at the bottom of the cell; part of the food is extruded backwards up between the descending blades and

undergo a shearing force (shown in the zoom image in Figure 1.7). When probe went down and compressed the samples packed solid, very small amount of compression force applied. Total work required during procedure were studied.

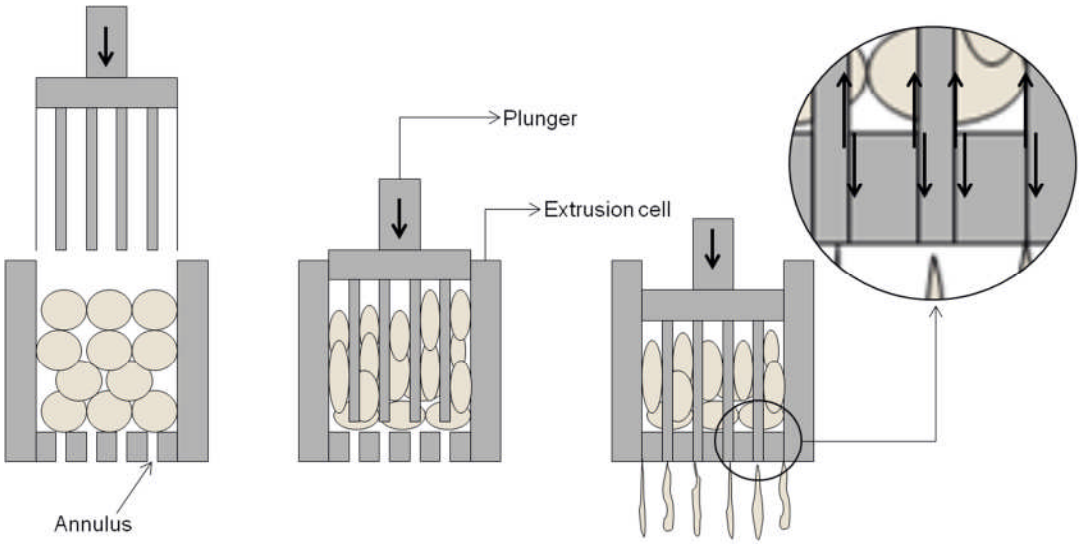

Figure 1.7 Schematic diagram of Kramer shear press measurement for a whole batch of navy bean (10g) using miniature Kramer shear cell.

1.3.3.2 Ottawa compression measurement

The standard cell of the Ottawa compression cell applies a combination of compression and extrusion forces but no or little shear. Constant amount (10g) of sample weight has to be used in this test too. Figure 1.8 shows the diagram to illustrate the procedure of Ottawa compression measurement for a whole batch of navy bean (10g) using a miniature Ottawa cell. The food is extruded forward through the slits in the bottom of the cell and applied the extrusion force. Also the food is compressed by the flat of the probe and applied the compression force. Total work required during procedure were studied.

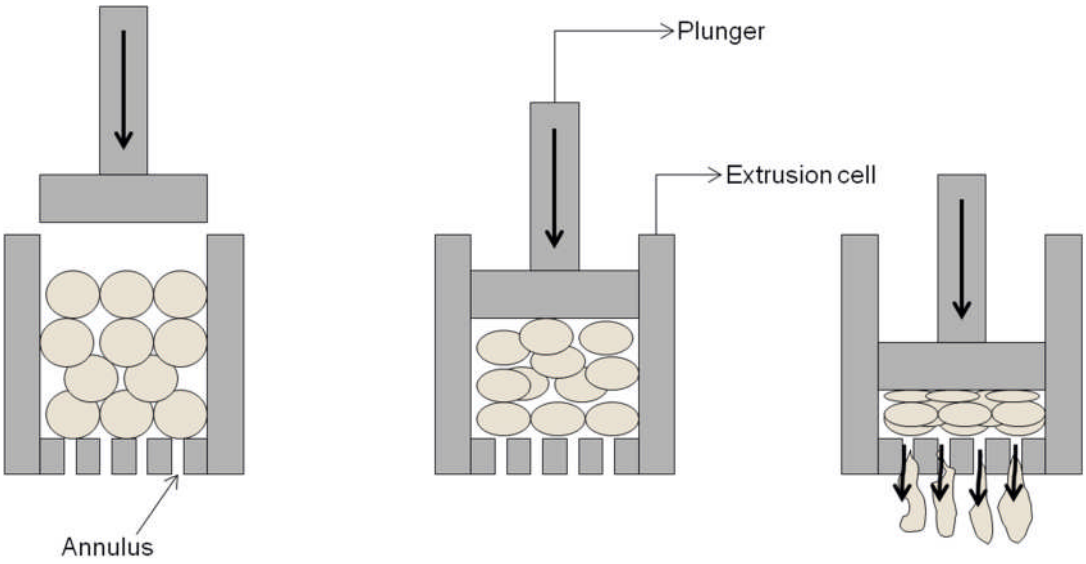

Figure 1.8 Schematic diagram of Ottawa compression measurement for a whole batch of navy bean (10g) using miniature Ottawa cell.

1.3.4 Viscosity measurement

Viscosity is the internal friction of a fluid or its tendency to resist flow, it is defined as the ratio of the stress over the rate of deformation. In general, layers of liquid move at different velocities in the flow and the viscosity arise from the shear stress between the layers. Temperature, concentration of solute, molecular weight of solute, suspended matter and pressure are considered as the factors affecting viscosity (Bourne, 2002). Rheological behaviour of fluids can be classed as Newtonian fluid flow and non-Newtonian fluid behaviour. Water and most gases are examples of Newtonian fluid, which is a fluid that has a constant viscosity over time at constant temperature and pressure. Non-Newtonian fluids are subdivided into time-independent fluids and time-dependent fluids (Figure 1.9). Time-dependent fluids include rheopectic liquids (increase in viscosity over time when shaken, agitated or stressed) and thixotropic liquids (decrease in viscosity over time when shaken, agitated or stressed with a constant shear rate) (Figure 1.9). Examples of time-dependent fluids are cream and yogurt whose structure changes over time due to rearrangement of colloidal structures. Figure 1. 10 shows the time-independent fluid viscous behaviour with varying shear rate. Shear thickening (dilatant) fluids increase in viscosity with the rate of shear, examples include a solution of corn starch in water, where hydrated starch molecules interact with each other over time. Shearing thinning (pseudoplastic) fluids decrease in viscosity with the rate of shear,

and it is the most common behaviour of biological liquids, such as ketchup and blood. Bingham plastic fluid is a material that behave as a rigid body at low shear stress, but flow as a viscous liquid at high shear stress, such as mayonnaise.

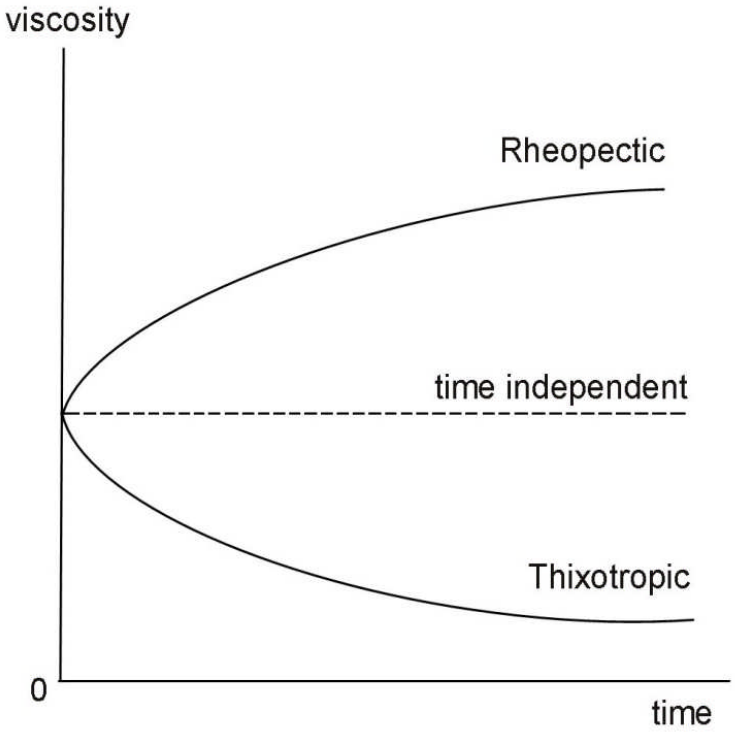

Figure 1.9. Schematic diagram time-dependent fluid classification.

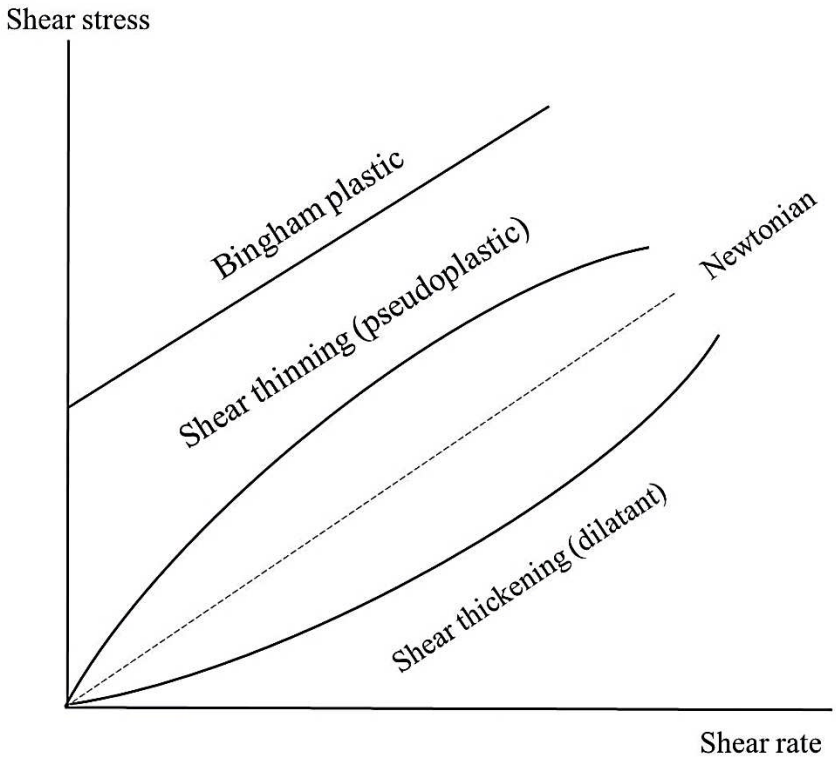

Figure 1. 10 Schematic diagram of time-independent fluid classification.

The canned sauce examined in this project belongs to category time-independent, shearing thinning fluids. The viscosity of the sauce was measured using a rheometer with axial geometry. Figure 1.11 illustrates the viscosity measurement of canned sauce in a typical rheometer. Canned sauce was poured into the cup, after starting the program, the bob (part of the geometry) went into the cup, rotated in one direction with increasing shearing rate which had been programed beforehand. The sauce was sheared by the rotating bob and the viscosity was measured at increasing shear rates. The viscosity is affected by both chemical composition and molecular size of the sauce components (Phatak et al., 1988). A typical sauce contains homogenised cooked tomato, modified starch, sugar and salt, the major component being the tomato. Tomato is rich in cell wall polysaccharides, most particularly pectins (Orfila and Knox, 2000). It is likely that the cell wall components, most particularly the polysaccharides, of the beans and the tomato sauce will have effects on the textural properties of the canned baked beans. The evolution of viscosity of tomato paste based products is suggested to be related to their mean volume diameter and tomato paste water insoluble solids content. The solid content is mainly controlled by tomato variety and tomato paste processing conditions (Valencia et al., 2002). The starch in the sauce may also influence the viscosity of

the sauce, because the modified starch in the sauce contained corn starch which is shear thickening material.

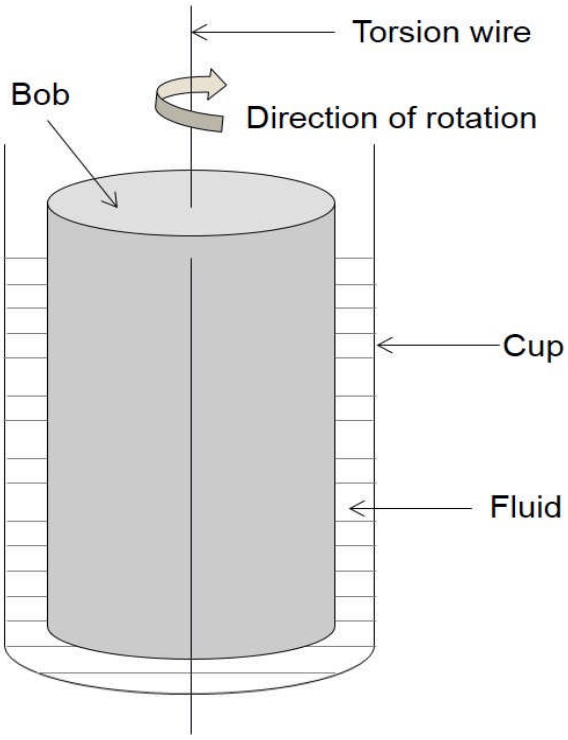

Figure 1.11. Schematic diagram of viscosity measurement of canned sauce in the axial geometry of the rheometer.

1.4 Cell wall

1.4.1 Cell wall biosynthesis and functionality

The cell wall of a plant is known to have many very important functions. Firstly, the cell wall regulates size, shape and strength to the cell and provides rigidity to the whole plant. Secondly, the cell wall is involved in the control of the cell growth by physically limiting the rate of expansion and division. Thirdly, the cell wall presents an obstacle to the movement of large molecules into and out of the cell. Furthermore, cell wall participates in cell – cell communication and influence interactions with herbivores through nutritional and health effects (Brett and Waldron, 1996, McNeil et al., 1984). The plant cell deposits its wall as a series of layers. Figure 1.12 shows the division of a typical plant cell and the formation of the primary cell wall. After mitosis, a new wall is laid down, establishing a boundary between two daughter nuclei. Small vesicles packed with non-cellulosic polysaccharides accumulate and align themselves in the plane of cell division, the vesicles fuse to form the earliest part of the new wall which is called cell plate, and later will become the middle lamella. The phragmoplast which lie perpendicular to the forming cell plate are thought to play a part in guiding vesicles to the edges of the cell plate (Fry, 1988, Brett and Waldron, 1996). Once the cell plate is completed and fuses with the pre-existing cell walls of the parent cell, the daughter cells proceeds to deposit the next major layer, the primary cell wall. Many plants limit themselves to the middle lamella and primary cell wall, certain specialized cells (e.g. fibre cells, vascular vessels) proceed to lay down a further wall layer over part of all of their surfaces, this is called the secondary wall (not shown in Figure 1.12).

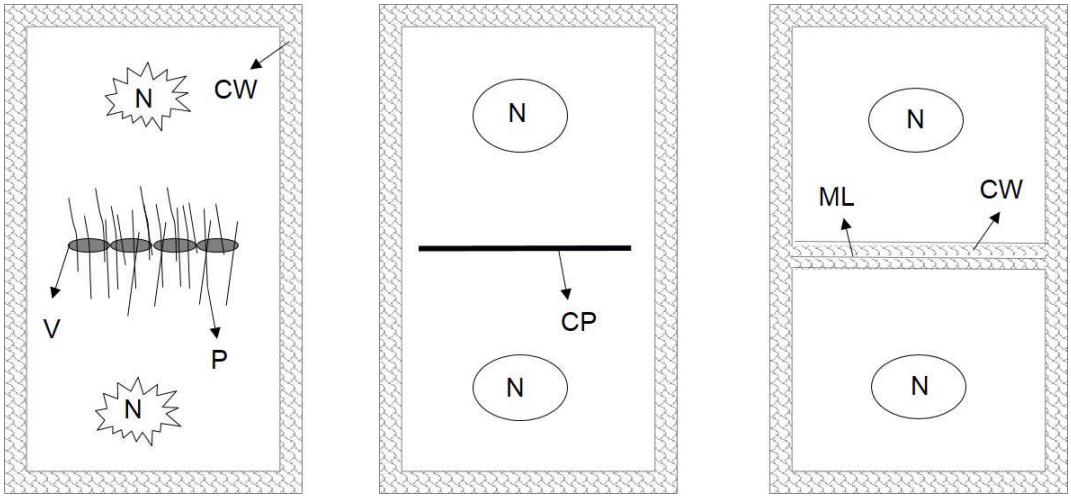

Figure 1.12 Schematic diagrams of stages in cell wall (CW) synthesis during cell division.

CW = cell wall; N = nuclei; V = vesicles; P = phragmoplast; CP = cell plate; ML = middle lamella.

1.4.2 Cell wall structure

Cell wall can consist of several layers, namely the middle lamella, the primary cell wall and the secondary cell wall (the latter only in specialised cells). Figure 1. 13 shows the plant cell wall structure and the pit field in the walls. The primary cell wall is deposited generally as a thin, flexible and extensible layer while the cell is increasing in size. The middle lamella is a pectin-rich layer that joins together the primary walls of adjacent cells, and is often the site of cell-to-cell adhesion. The secondary cell wall is deposited as a thick layer formed inside the primary cell wall and is largely impermeable. For this reason, channels between cells exist, called pits which form in sections where the cell wall is missing. In the primary cell wall, there are also cluster of holes. These holes are called plasmodesmata. A cluster of plasmodesmata is called a primary pit field (Raven et al., 2012). Plasmodesmata and pits allow direct cell to cell communication between cytoplasm of adjacent cells. The primary and secondary walls have different functions reflected in their architecture and composition. Primary walls have to resist catastrophic bursting in the face of turgor pressure of several atmospheres, meanwhile be plastic enough to allow an appropriate extent of growth. Secondary walls are very rigid and impermeable, and play important roles including defence, support and storage (Fry, 1988).

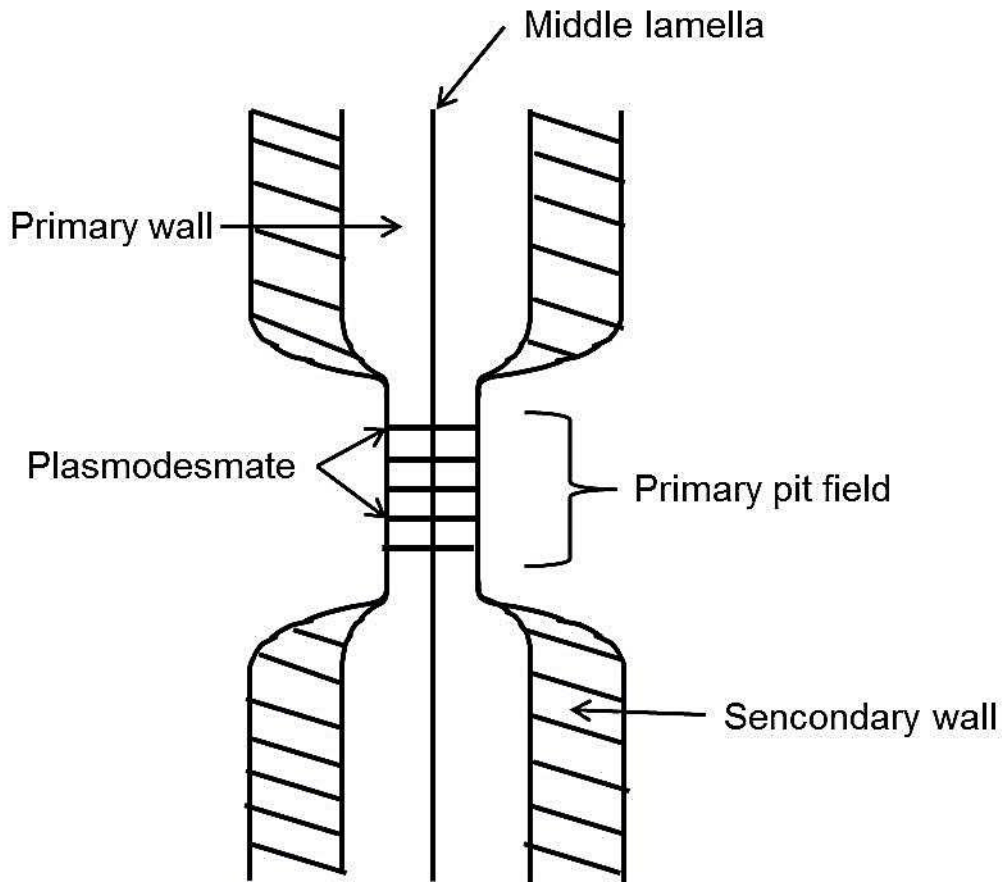

Figure 1. 13 Schematic diagrams of plant cell walls and pit field.

In this project, primary cell walls were the focus of investigation as pectic polysaccharides are mainly exist in the primary wall (Brett and Waldron, 1996), secondary cell wall was not investigated. Figure 1.14 shows the schematic diagram showing the structure and the composition of primary cell walls. Cellulose microfibrils are arranged into parallel structures, cross-linked into a rigid network by hemicellulose molecules. The hemicellulose molecules are linked to the surface of the microfibrils by hydrogen bonds. The cellulose – hemicellulose network is penetrated by a network of pectins. The middle lamella is a pectin-rich layer that glue adjacent cells together (Raven et al., 2012). Heat treatment was found to influence the structure of the cell wall, causing partial disassembly of the cell wall and middle lamella (Stolle-Smits et al., 1998). Hence, it is hypothesised that the processing may have significant effects on the microstructure of the cell wall during canning of beans.

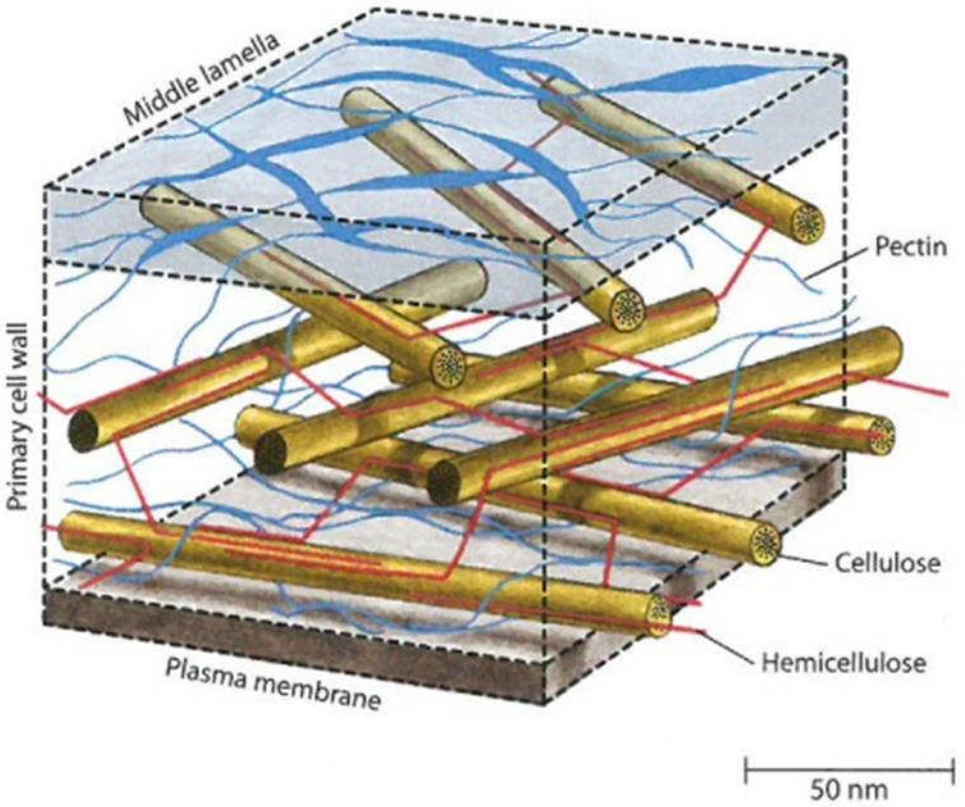

Figure 1.14 Schematic diagram showing the structure and the composition of primary cell walls (Raven et al., 2012).

1.4.3 Primary cell wall chemical composition

Primary cell walls consist of a microfibrillar phase and a matrix phase. The microfibrillar phase is composed of cellulose and cross linking hemicelluoses. The matrix phase consists of pectins, hemicelluloses, proteins and phenolic compounds (Brett and Waldron, 1996). Table 1.2 shows the typical composition of primary cell walls. In legumes, phenolic contribute a very small portion (approximately 0.5% - 1%, w/w %) but may have significant effects on strength through phenolic crosslinking. This is followed by cell wall proteins which contribute approximately 15% w/w % of cell wall (Srisuma et al., 1991, Shiga et al., 2004, Kereliuk and Kozub, 1995). Proteins can also contribute to cell wall strength through covalent and other interactions. The bulk of the cell wall components (approximately 85%, w/w % of cell wall) are cell wall polysaccharides, including cellulose, hemicellulose and pectic polysaccharides. Cell wall polysaccharides can be hydrolysed into monosaccharides using acid at high temperature. Figure 1.15 shows the structure of monosaccharides commonly found in plant cell walls, including D-glucose, D-

mannose, D-galactose, L-arabinose, L-rhamnose, L-fucose, D-xylose, D-galacturonic acid and D-glucuronic acid. Cell wall polysaccharides are through to play a dominant role in cell wall physiology, they not only determine the cell size and morphology, but also affect the rate of cell growth and activate the mechanism for resistance to potential pathogens (McNeil et al., 1984). The solubilisation and depolymerisation of polymers were found to be responsible for tissue softening of food during cooking (Brett and Waldron, 1996, Liu, 1995). Beans texture was also found to associate with the solubility of cell wall polysaccharides, harder beans showed reduced solubility of cell wall polysaccharides compared to softer beans (Shiga et al., 2004). Therefore, the cell wall polysaccharides may have significant effect on the texture of the canned beans.

Table 1.2. The compositions of cell wall.

Phase	Components	
Microfibrillar	Cellulose (β 1, 4 – glucan)	
Matrix	Pectins (Pectic polysaccharides)	Rhamnogagcturonan I
		Arabinan
		Galactan
		Arabinogalactan I
		Homogalacturonan
		Rhamnogalacturonan II
	Hemicelluloses (some tightly associated with cellulose)	Xylan
		glucomannan
		Mannan
		Galactomannan
		Glucuronomannan
		Xyloglucan
		Callose (β 1, 3 – glucan)
		B 1, 3 -, β 1, 4 – glucan
		Arabinogalactan II
	Proteins	Extensin
		Arabinogalactan – proteins
		Others, including enzymes
	Phenolic	Ligin
		Ferulic acid
		Others, e.g coumaric acid, truxillic acid

(Adapted from (Brett and Waldron 1996))

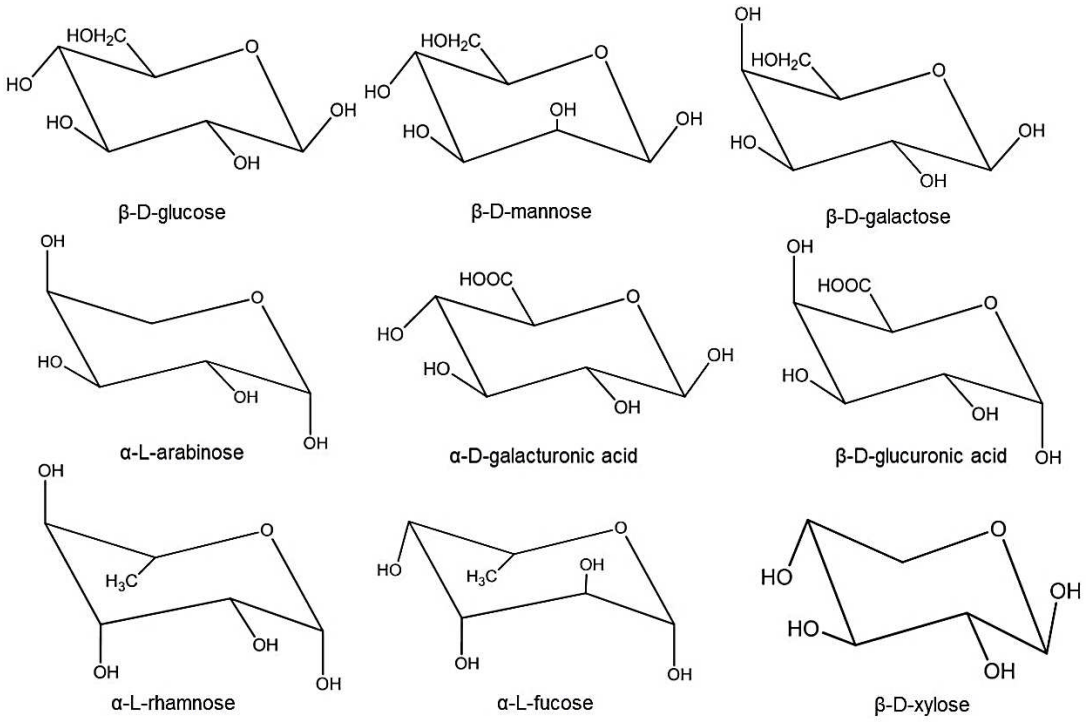

Figure 1.15 Structure of monosaccharides commonly found in plant cell wall

1.4.3.1 Cellulose

Cellulose is the main component of the cell walls, contributing approximately 30% (dry basis) of the total cell wall weight in the beans (Srisuma et al., 1991). Cellulose is an unbranched β 1, 4 – glucan with a DP (degree of polymerization, number of sugar residues per molecule) of at least 15000 (Brett and Waldron, 1996). Figure 1.16 shows the structure of a cellulose microfibril. The cellulose molecules assemble into the microfibrils which are circular or oval in cross section. The cellulose molecule align parallel along the axis of the microfibril. The cellulose chains assemble into a crystalline structure within the microfibril, and the lattice is stabilized by both intramolecular and intermolecular hydrogen bonds, excluding water. However, the microfibril may consist of a crystalline core surrounded by a rather less crystalline surface region where hemicelluloses can interact (Brett and Waldron, 1996).

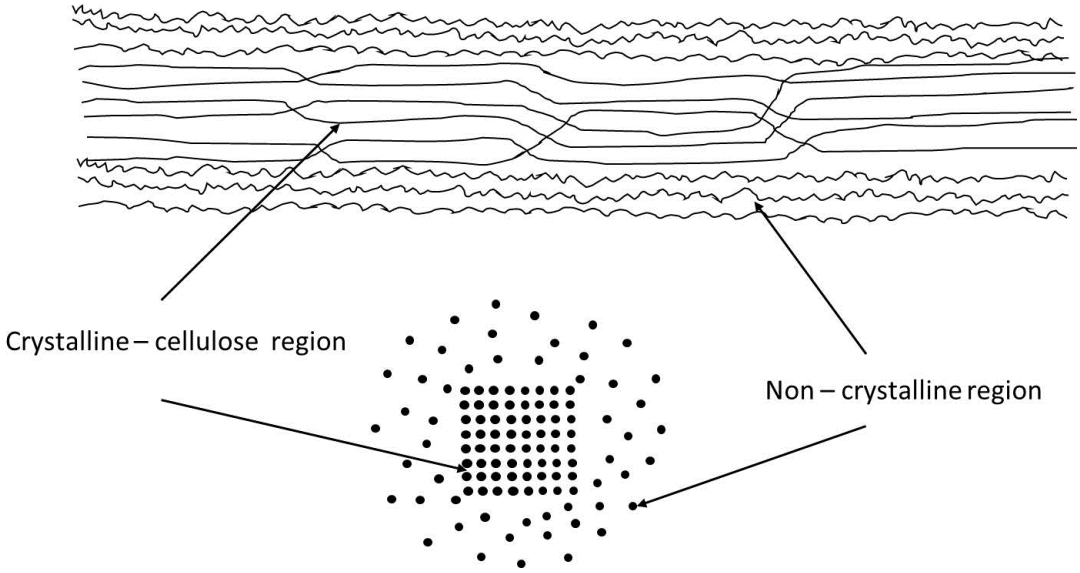

Figure 1.16 Schematic diagrams of cellulose microfibrils. Showing the crystalline and non – crystalline region. The non – crystalline region contain both cellulosic and non – cellulosic polysaccharides (Adapted from (Brett and Waldron, 1996)).

1.4.3.2 Hemicelluloses

Hemicelluloses contributes approximately 20% of the total cell wall content in the beans (Srisuma et al., 1991). Hemicelluloses are polysaccharides that have β 1, 4 - linked backbones, but not necessarily of glucose. They consist of shorter chains than cellulose (500 – 3000 contrast to up to 15000 for cellulose). Hemicelluloses includes xylans, xyloglucans, arabinoxylans, mannans and glucomannans (Walter and Taylor, 1991). So the monosaccharides in hemicellulose can include xylose, mannose, galactose, rhamnose and arabinose. The most abundant hemicellulose component in the primary cell wall of dicotyledonous species are xyloglucans, other classes also include xylan and mannan (Orfila, 2001). The chains of xyloglucan can form hydrogen bonds with non-crystalline cellulose chains, at the surface or the edge of the cellulose microfibrils cross linking several cellulose fibrils (McCan et al., 1990). Breakdown of hemicellulose polymers accompany fruit softening in some species such as melon and kiwi.

1.4.3.3 Pectin

Pectin, also called pectic polysaccharide, is abundant in primary cell walls and in middle lamella, contributing to approximately 30% - 35% to the total cell wall compositions in the beans (Srisuma et al., 1991, Mohnen, 2008). Pectins are the most complex polysaccharides structurally and functionally in the cell wall (Mohnen, 2008). Because of the complicated structure of pectins, it is suggested that they have multiple functions. Pectins are claimed to contribute to the cell wall structural integrity, cell adhesion and mediation of defence responses (Caffall and Mohnen, 2009). In the legume seeds, tissue softening seems to be achieved through the solubilisation of pectins from the middle lamella, which allows cell separation (Ilker and Szczesniak, 1990). The amount of soluble pectin in various beans was associated with the firmness of cooked beans (Wang and Chang, 1988). Lu and Chang (1996) also indicated that larger amount of soluble pectin decreased the firmness of the navy beans. Therefore, it is believed that pectin may have an important influence on the texture quality of canned baked beans.

Pectic polysaccharides are a family of complex polysaccharides. Figure 1.17 shows a diagrammatic representation of the structure of pectin. Pectin can be fractioned into acidic polysaccharides including homogalacturonan (HG), rhamnogalacturonan I (RGI), rhamnogalacturonan II (RGII) and xylogalacturonans (XGA) (Willats et al., 2001a, Scheller et al., 2007, Mohnen, 2008). HG, RGI, RGII and XGA polymers are covalently linked to each other and rich in uronic acid (approximately 70% of pectin) (Mohnen, 2008, Ishii and Matsunaga, 2001, Caffall and Mohnen, 2009). Un-methyl-esterified HG are cross linked to each other by calcium (Figure 1.17). Besides the acidic polysaccharides, pectin also contains neutral oligosaccharide or polysaccharide chains such as arabinans, galactans and arabinogalactans which may be covalently attached to acidic polysaccharides (Knox et al., 1990). The presence of the polysaccharides differ between cell types and species in terms of their relative abundance and structural details (Scheller et al., 2007). In fact, the composition may vary within a given cell wall. In this project, HG and side chain of RGI were investigated.

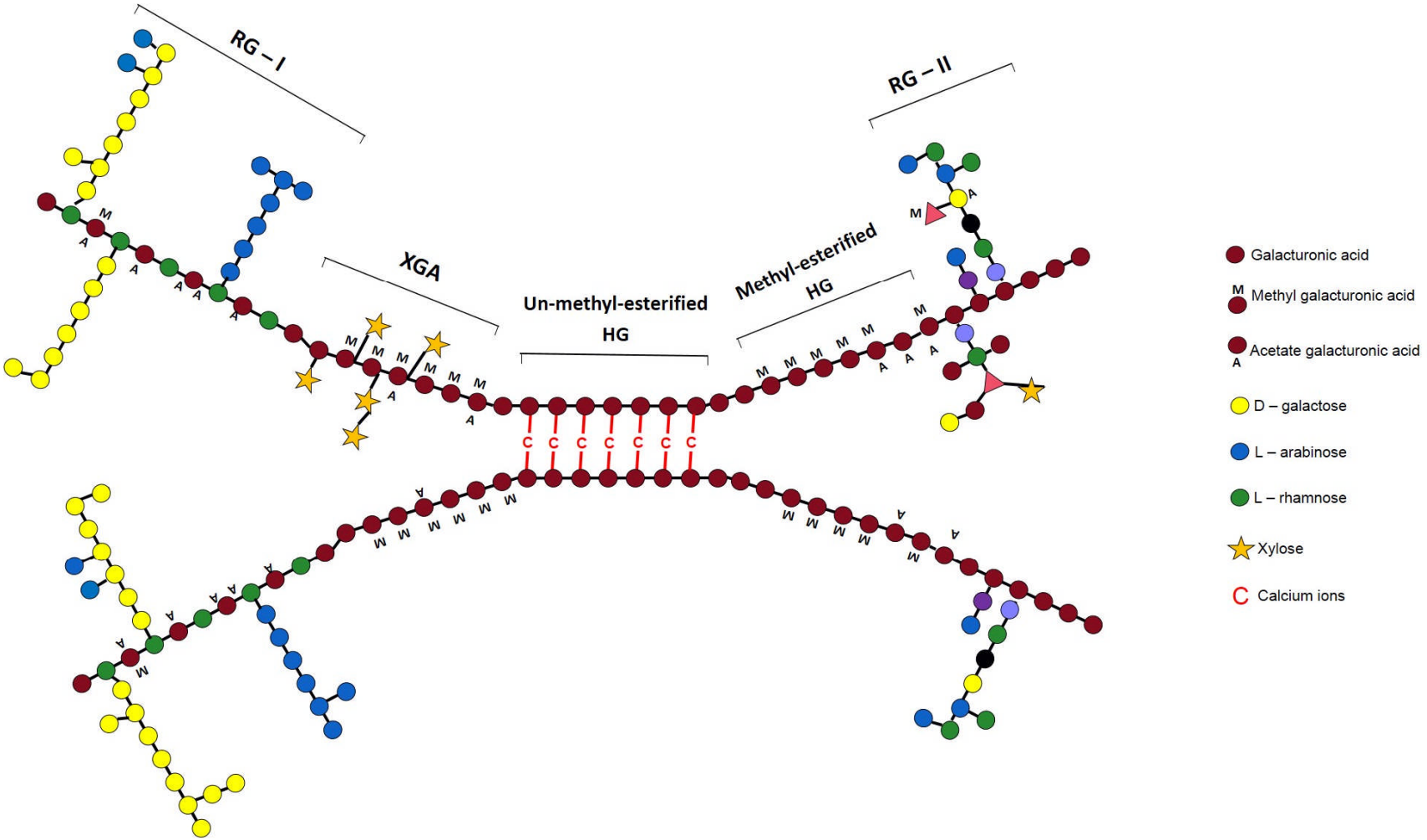

Figure 1.17. Schematic diagram of the structure of pectin (pectic polysaccharides).

i. Homogalacturonan (HG)

HG is the most abundant pectic polysaccharide comprising approximately 60% - 65% of the pectin (Mohnen, 2008, Caffall and Mohnen, 2009). It is a linear polysaccharides with a backbone of α-1, 4-linked galacturonic acid residues (Mohnen, 2008). Some studies have indicated that HG is usually synthesised in a largely methyl-esterified form in the Golgi apparatus and some of them can be de-esterified in the cell wall by the enzyme pectin methylesterases (PMEs) (Clausen et al., 2003). Thus, there are methyl-esterified HG and un-methyl-esterified HG in the cell wall. The methyl-esterified HG is usually methylesterified on C6. It can also be acetylated on C2 or C3 (Scheller et al., 2007, Mohnen, 2008). The un-methyl-esterified HG can form calcium crosslinks to each other and bring blocks of un-methyl-esterified HG chains into tightly packed conformations, consequently contributes to the wall strength (Caffall and Mohnen, 2009). Figure 1.18 shows the egg-box model of the calcium crosslinking of un-methyl-esterified HG. The egg-box model describes how the packing of HG occurs upon Ca^{2+} binding, the un-methylated C6 of galacturonic acid is negatively charged and ionically interact with Ca^{2+} (Caffall and Mohnen, 2009). It has been shown that HG in the middle lamella is largely unesterified and may play important in forming cell to cell adhesion sites (Mohnen, 2008).

HG is an abundant and multifunctional matrix polymer in all primary cell walls, it plays an important role in growth, development, defence and cell adhesion (Manfield et al., 2005, Clausen et al., 2003). Stolle-Smints et al. (1999) studied the bean pod and indicated that the increase in HG-calcium complexes in the wall would increase cell wall stiffening and decline the wall expansibility. (Stolle-Smits et al., 1999). HG-calcium complexes (egg-box model) can result in gel formation, and contribute to cell wall strength, cell intercellular adhesion and stomatal function.(Caffall and Mohnen, 2009, Willats et al., 2001b)

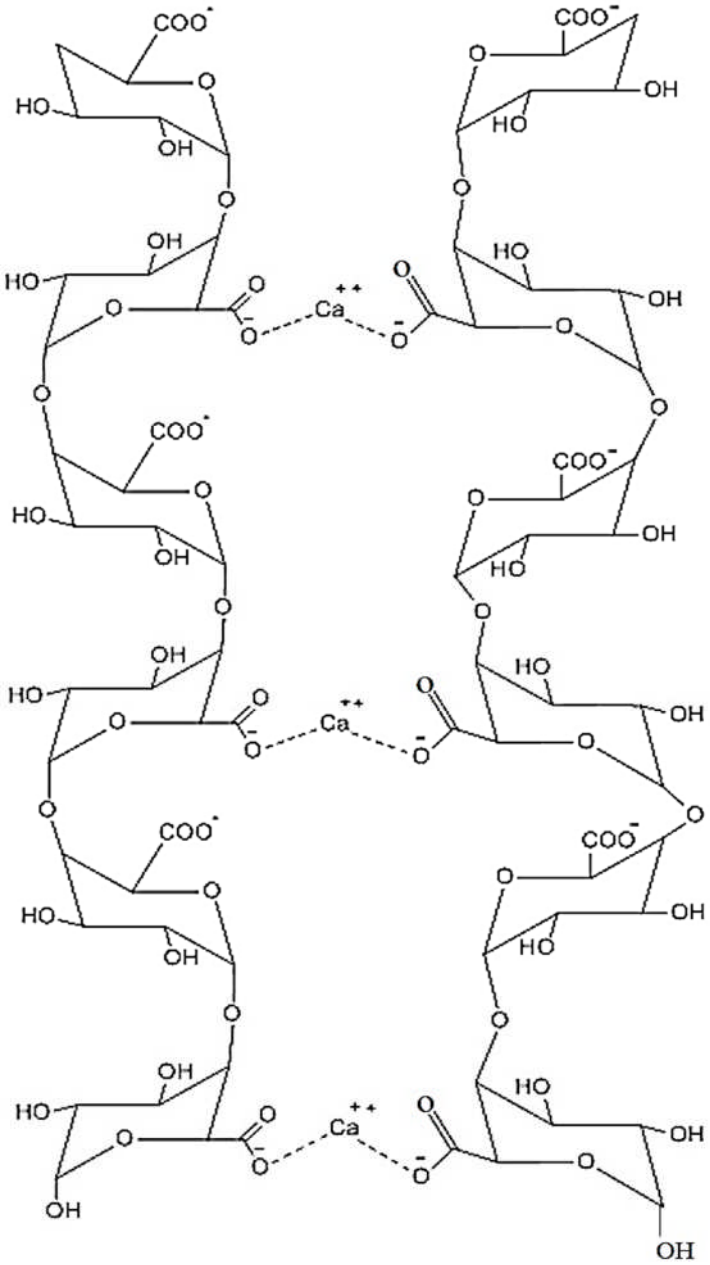

Figure 1.18 The egg-box model of calcium crosslinking in un-methyl-esterified HG polysaccharides (Adapted from (Caffall and Mohnen, 2009)).

ii. Rhamnogalacturonan I (RGI)

RGI makes up 20% - 35% of pectin (Mohnen, 2008). It consists of a backbone which is often substituted with side chains. RGI's backbone is made of alternating covalent linked rhamnose and galacturonic acid residues, with 20% - 80% of the rhamnose residues substituted with (1-4)-β-galactans, (1-5)-α-L-arabinans or arabinogalactans (Albersheim et al., 1996). These side chains can be linear or branched arabinan, galactan or arabinogalactan (Mohnen, 2008, Scheller et al., 2007).

The side chains are complex and variable (Mohnen, 2008), they may be cross linked to other wall components such as hemicellulose, proteins and phenolic compounds (Caffall and Mohnen, 2009, O'Neill and York, 2003). Galactans are mostly linear chains of β-1,4-linked galactose residues, arabinan are chains of α-1,5 linked arabinofuranose residues that can carry branches of arabinan at O3 or O2. Arabinogalactan I side chains are generally β-1,4-galactan chains with arabinan branches, Arabinogalactans II side chains are more complex and are often associated with proteins(Mohnen, 2008, Scheller et al., 2007).

RG I side chains were found to influence cell adhesion (Caffall and Mohnen, 2009). Stolle-Smints et al. (1999) claimed that in bean pod, a decrease in RGI neutral sugar side chains was associated with an increase in wall stiffening and decline in wall expansibility. The degradation of arabinan and galactan coincided with the de-methylation of the HG by PME, and subsequently the formation of the HG-calcium complex, which contribute the cell wall strength. This indicates that RGI may regulate the access of enzymes to their substrate in the cell wall. Navy beans (Phaseolus vulgaris L.) were found to contain high amount of arabinan and medium amount of galactan (Shiga et al., 2004). Therefore, it is possible the arabinan and galactan content may contribute to the texture characteristic of the beans.

iii. Monoclonal antibody

In recent years, a number of anti-pectin monoclonal antibodies have been generated and used to study pectin. Monoclonal antibodies are powerful tools for targeting a small fraction of molecules against a complex background (Manfield et al., 2005). Several studies have used monoclonal antibodies to localise the pectin epitopes at the level of individual cell wall, in order to visualise the microstructure of the cell wall, and their spatial changes during plant development. This approach had successfully been used to study the role of pectins as determinants of texture in tomato, pea root and pea cotyledon (Knox, 1997, Ordaz-Ortiz et al., 2009, Orfila et al., 2001, McCartney et al., 2000). Monoclonal antibodies were developed in response to immunization of rats with protoplasts prepared from plant cells but recognize antigens of the cell wall rather than the plasma membrane. In this project, four probes were selected to investigate the pectin in the cell wall of navy beans, they are JIM5, JIM7, LM5 and LM6. When studying the microstructure of beans,

LM21 and LM1 were also used to investigate mannans and cell wall protein in the cell wall.

a) JIM5

JIM5 was raised in rat against a carrot protoplast preparation (Vandenbosch et al., 1989) and recognises a relatively un-esterified epitope of homogalacturonan (HG) (Vandenbosch et al., 1989, Knox et al., 1990). JIM5 binds to at least four contiguous unesterified residues between or adjacent to a methyl-ester group (Figure 1.19), JIM5 binds in preference to HG with a relatively low level of methyl-esterification or fully unesterified HG (Willats et al., 2000).

b) JIM7

JIM7 was raised in rat against a carrot protoplast preparation and recognises methyl-esterified epitope of HG (Knox et al., 1990). JIM7 epitope are methyl-ester groups at every second residue and no preference for the esterification state of the intervening residue (Figure 1.19) (Clausen et al., 2003). JIM7 binds to a range of HG epitopes with a relatively high level of methyl-esterification, but not completely de-esterified HG (Willats et al., 2000).

In conclusion, JIM5 optimally binds to unesterified GalA residues with adjacent or flanking methyl-esterified residues or fully unesterified residues. In contrast, JIM7 binds to methyl-esterified residues with adjacent or flanking unesterified GalA residues (Clausen et al., 2003).

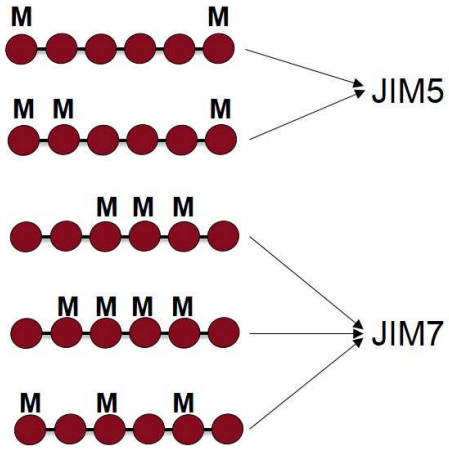

Figure 1.19 Antibodies JIM5 and JIM7 binds to the methyl hexagalacturonates with different presence or absence of methyl-ester groups. (Adapted from (Clausen et al., 2003))

c) LM5

LM5 was raised in rat against a synthetic neoglycopotein consisting of the oligosaccharide [(1-4)-β-D-Galρ]$_4$ conjugated to bovine serum albumin (BSA; Jones et al.,1997). LM5 binds to galactan, which is the side chain of RGI (McCartney and Knox, 2002)

d) LM6

LM6 was raised in rat against a synthetic neoglycopotein consisting of the oligosaccharide [(1-5)-α-L-Araf]$_7$ conjugated to BSA (Willats et al., 1998). LM6 binds to arabinan, which is the side chain of RGI (McCartney and Knox, 2002)

e) LM21

Monoclonal antibody LM21 has been claimed to have affinity for heteromannan (Marcus et al., 2010). Heteromannan is a form of mannan which consists of two or more different monomers of mannose, galactose or glucose. LM21 was found to bind to mannan polysaccharides in the primary cell wall, and secondary wall of some legumes (Marcus et al., 2010, Albersheim et al., 2011).

f) LM1

Monoclonal antibody LM1 has been claimed to have affinity for extensin (Smallwood et al., 1995). Extensin is known to be abundant in dicotyledonous primary cell walls, and it is a family of hydroxyproline-rich glycoproteins (HRGPs) (Smallwood et al., 1995).

1.5 Dietary fibre

All cell wall components are also part of what is called ‘dietary fibre’. Many analytical techniques have been develop to isolate dietary fibre from foods, and these preparations will largely contain (although not exclusively) cell wall components.

Dietary fibre is an essential nutrient in navy beans which helps people with defecation. Investigation of the dietary fibre content is a way for us to study the cell wall polysaccharides in the navy beans. Table 1.3 shows the association between dietary fibre and cell wall polysaccharides according to the Association of Organic Analytical Chemists (AOAC) method. Dietary fibre consists of non-starch

polysaccharides, lignin, and resistant starch and protein. The non-starch polysaccharides (including cellulose, hemicellulose, pectin and other polysaccharides) lignin and some resistant proteins are derived from cell wall material. Non-starch polysaccharides, including pectin, hemicellulose, and cellulose, are the major constituent of dietary fibre in plant food (Selvendran, 1984). Therefore, in this project, the cell wall materials were isolated using methodology designed to isolate dietary fibre.

Table 1.3 Constituents of dietary fibre measured by the Association of Organic Analytical Chemists (AOAC) method.

<table>
<tr><td rowspan="6">AOAC – fibre</td><td rowspan="4">Non-starch polysaccharides</td><td rowspan="3">Non-cellulosic polysaccharides</td><td>Pectin</td><td rowspan="4">Cell wall</td></tr>
<tr><td>Hemicellulose</td></tr>
<tr><td>Other polysaccharides</td></tr>
<tr><td colspan="2">Cellulose</td></tr>
<tr><td colspan="3">Lignin</td></tr>
<tr><td colspan="4">Resistant starch</td></tr>
<tr><td colspan="4">Other un-digestible materials</td></tr>
<tr><td colspan="5">Oligosaccharides (e.g. raffinose, stachyose)</td></tr>
</table>

(Adapted from British Nutrition Foundation (1990))

Dietary fibre is the un-digestible portion in the food. Dietary fibre can be categorised as water insoluble polysaccharides (WIP) and water soluble polysaccharides (WSP). WIP contains cellulose, hemicellulose and other insoluble polysaccharides which provided ‘roughage’ to provide bulk in the human colon. WSP contains soluble oligosaccharides and polysaccharides which are often capable of forming gels or thick solutions and help slow down the digestion process and transit time and are readily be fermented in human colon to forms gases.

Navy beans were found to have a high level of dietary fibre content among the legumes (up to 26%, w/w% dry weight basis), 9% - 23% (w/w% dry weight basis) was WIP and 3% - 6% (w/w% dry weight basis) was WSP (Costa et al., 2006, Shiga and Lajolo, 2006, Kereliuk and Kozub, 1995). Srisuma et al. (1991) studied the fibre content of raw navy beans, they claimed that bean skin (seed coat) cell wall was especially rich in cellulosic polysaccharides (range 58.7% - 65%, approximately two third of skin cell wall) which indicating its strong physical property to support its biological function as a protective tissue, whereas the more flexible structure of the cotyledon cell wall was composed principally of matrix polysaccharides and was especially rich in WSP (25.7% - 32.5% of cotyledon cell wall) compared to skin, and cellulose made up approximately one third of cotyledon cell walls (range 30.6% - 34.6%).

Therefore, the previous study indicated that navy beans contained high level of cell wall material, which can potentially contribute to the bean's physical, mechanical and biochemical properties. In this project, the dietary fibre content and its composition will be studied in order to investigate the role of cell wall in determining the texture of canned baked beans.

1.6 Starch

Starch is a homopolymer consisting of a large number of glucose units. The glucose units are joined into two types of polysaccharide: amylopectin and amylose. Amylose has a linear structure of α-1,4 glucose, and often is the minor component of starch in most plant, contributing approximately 25% of the total starch. The average DP (degree of polymerization) of legume amylose is 1000-1900, and navy bean amylose DP is 1300, which is much lower DP compared to potato (DP=3200) (Biliaderis et al., 1981). Amylopectin is larger than amylose and composed approximately 75% of the starch. It consists of a backbone of α-1,4 glucose with α-1,6-branched glucose side chains. The average CL (chain length) of legume amylopectin is 19 – 34, and navy bean amylopectin CL is 22. Each chain consist of a linear material with DP range from 14 – 45 (Biliaderis et al., 1981). Starch is the most abundant carbohydrate in the legume seeds. Legumes such as lentils, pinto, chickpea contain 38% - 48% starch out of 50% - 60% carbohydrate (Rehman and Shah, 2005, Berg et al., 2012). Starch in legumes is found in starch granules in the

cotyledon cells (Hahn et al., 1977), and are embedded in the protein matrix of the cellular contents (Daussant et al., 1983)

Navy beans have a higher level of amylose content, amylopectin average chain length, proportion of long amylopectin branch chains (DP≥37), and resistant starch (RS) compared to pea and lentil starches (Chung et al., 2010).The cotyledon cell of navy beans was shown to be hexagonal or angular shape (50 – 100 μm size) containing starch granules with a size ranging between 10 and 50μm, they swelled and gelatinized during cooking (Berg et al., 2012). The thick and mechanically resistant nature of the cotyledon cell walls in legumes prevent complete swelling of starch granules during gelatinization which may restrict their interaction with digestive enzymes (Wiirsch et al., 1986). This restriction may also influence the osmotic potential of the starch during cooking.

1.7 Other factors affecting the texture of navy bean

Apart from the biochemical and structure factors mentioned above, other components in the beans may also have effect on the texture of the beans, such as protein, phenolics and saponins. Navy beans were found contain 23% - 27% (w/w%, dry weight basis) protein (Kereliuk and Kozub, 1995). Navy beans contained small amount of phenolic acid content including ferulic, coumaric, sinapic and cinnamic (25.74mg/100g bean) (Drumm et al., 1990). The functionalities of these components for navy beans were not well known, and because of time limitations, were not studied in this project.

The quality of canned beans is known to be affected by several factors, such as cultivars, growth regions, process conditions and storage conditions (Hosfield et al., 1984, Nordstrom and Sistrunk, 1977, Junek et al., 1980, Shiga et al., 2004). Cultivar or region was found to have significant effect on the physical (water imbibition), mechanical (firmness), and chemical properties of navy beans (Lu and Chang, 1996, Wang et al., 1988, Srisuma et al., 1991, Kereliuk and Kozub, 1995). Therefore, these factors will be studied in this project.

1.8 Outline of this thesis

1.8.1 Hypothesis

i. The cell wall structure and composition are the factors that determine the texture quality of canned baked beans.

ii. Cultivar, growth region, thermal processing has a significant effect on the mechanical and biochemical properties of canned baked beans.

1.8.2 Aims and Objectives

i. To assess and compare the mechanical and textural properties of selected bean from different cultivars and growth regions, using instrumental physical measurements (Texture Analyser). This will allow the classification of cultivars and growth regions into ‘firm’ or ‘soft’ varieties. Commercial varieties commonly used by Heinz will be assessed.

ii. To investigate the structure and composition of the cell wall polysaccharides of navy beans, using a combination of analytical and cytochemical techniques. Analytical techniques include monosaccharide composition analysis of cell wall preparations by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). Selective extraction of polymers from the cell wall using chemical agents or enzymes can be used to understand the solubility and composition of the different types of cell wall polymers (Obro et al., 2004, Orfila et al., 2002). The spatial distribution of cell wall polysaccharides will be investigated using immunofluorescence microscopy of bean sections using monoclonal antibodies that specifically recognise the pectin in cell wall.

iii. To investigate the effects of processing treatments (blanching and canning) on the mechanical, structural and chemical properties of beans. This will be done by simulating the canning process in the Food Technology laboratory and Heinz industry.

iv. To investigate the effects of cultivar and growth region on the physical, mechanical, structural and chemical properties. Seventeen types of beans were obtained from commercial procucer, which included 9 cultivars and 5 growth regions. The cultivar and region observation will give the industry a

better and direct guide to obtain the ‘optimum’ beans which match the customer’s requirement under the commercial consideration.

v. To develop methodology based on antibody recognition of soluble polysaccharides to assess and predict textural quality. The methodology will be based on ELISA.

vi. To compare the methods developed in terms of quality of data, robustness, ease of use and cost effectiveness.

Chapter 2
Materials and Methods

2.1 Plant material

Samples of this project were navy beans obtained from commercial bean producer. There were a total of seventeen samples from five growth regions and eight cultivars (Table 2.1). Beans from China were commercial batches of unknown mix varieties. Four main regions were A, B, C and D in North America shown in Figure 2.1. Region A and B is located in the centre of North America, it is a region that has an extreme climate characterised by high humidity in the short summer to an extreme temperature of 53.0°C and extremely cold and long winter with temperatures as low as −40°C. Region C located in the east-central part of Canada, and most of region C's climate is classified as humid continental, with warm to hot (and often very humid) summers at average temperature 28 °C, and cold winters at average temperature -5 °C. Region D located in the upper Midwestern region of United States of America, it has a typical of continental climate, with hot semi-humid summers at average temperature 30 °C, and cold dry winters at average temperature -10 °C. A flow chat of how the beans were treated before analysis is shown in Figure 2.2. Beans were grown by the suppliers during April to June (2011), and then harvested during July to September; they were stored in outdoor silos until February, and sent by ship to the UK in 2 ton bags on March. The beans are then stored in warehouses until processed.

Table 2.1 Table of the bean samples.

Labelled bean name	Growth region	Cultivar
A1	**A**	**1**
B1	**B**	**1**
C1	**C**	**1**
D1	**D**	**1**
A2	**A**	**2**
B2	**B**	**2**
C2	**C**	**2**
D2	**D**	**2**
B3	B	3
D6	D	6
D7	D	7
C5	C	5
C4	C	4
E5	A	5
F8	D	8
Ch09	China	N/A
Ch10	China	N/A

N/A not available

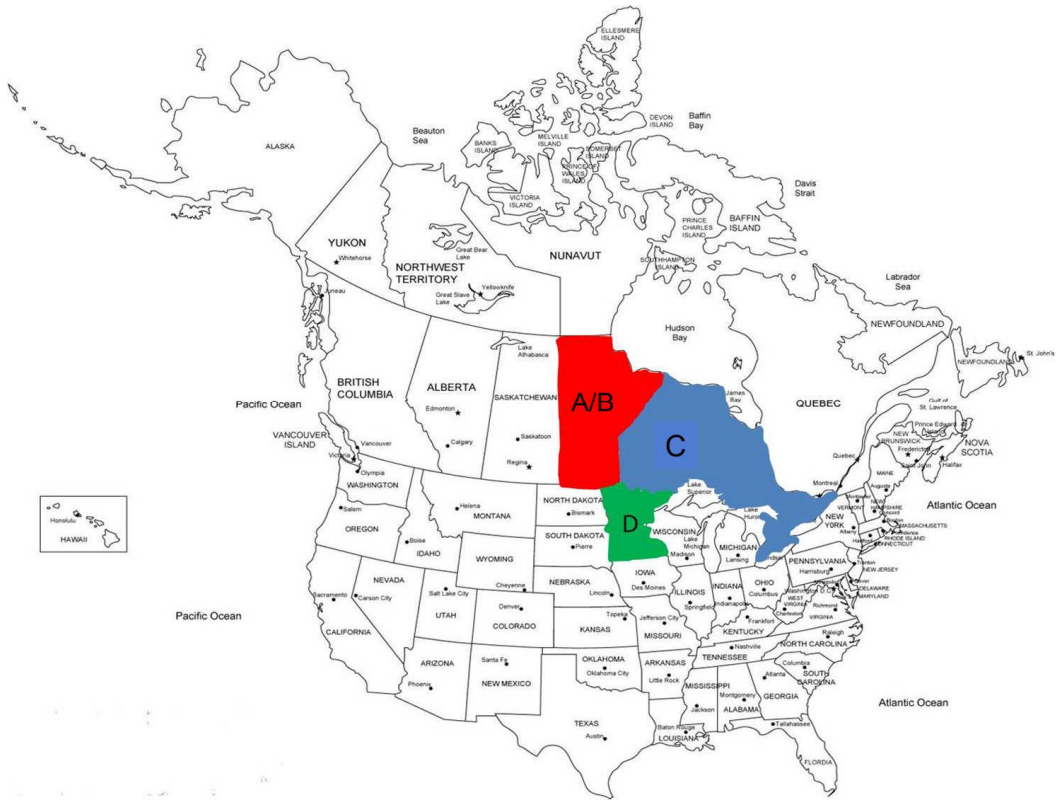

Figure 2.1 Map of the growth region of navy beans in North America.

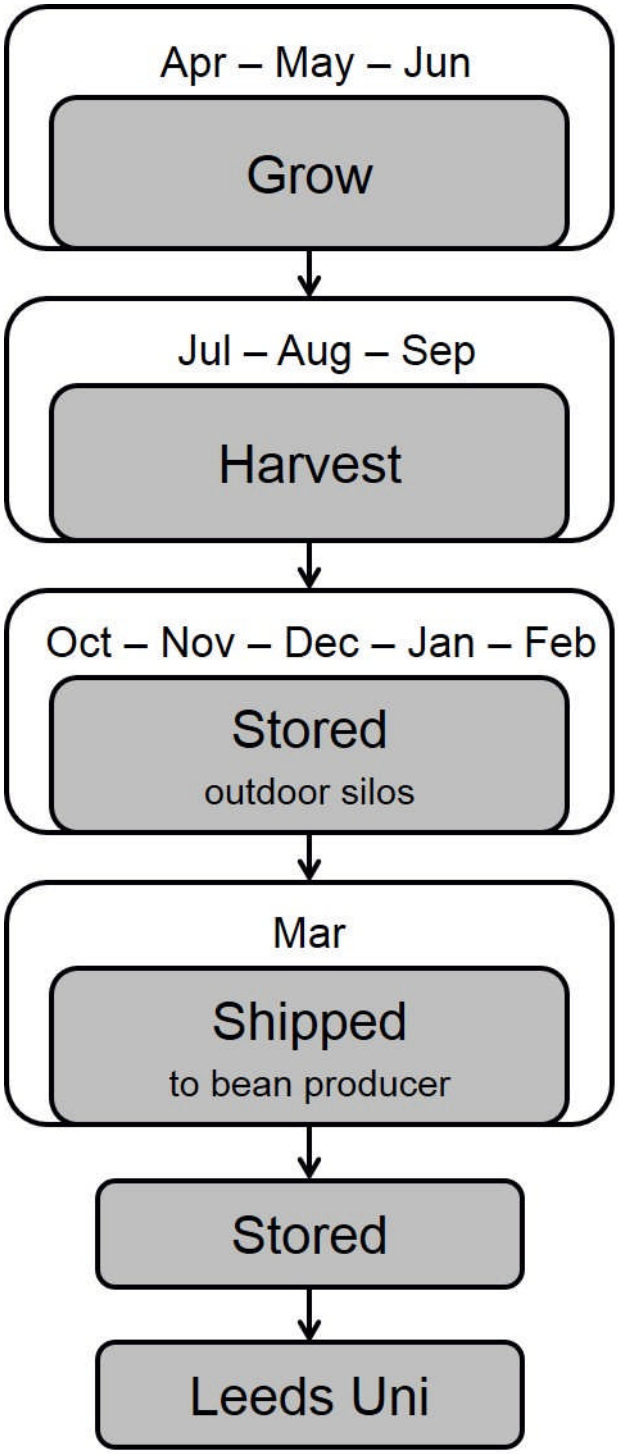

Figure 2.2 Flow chart of the growth, shipping and storage of navy beans

2.1.1 Sample processing

Laboratory processing:

Samples were processed as blanching and canning. Blanched beans were blanched in laboratory at 85°C for 20 min using tap water. Blanched beans and blanched water were collected for analysing. Canned beans were canned in laboratory retort for 21min at 126°C.

Industrial processing:

Canned beans were obtained from commercial bean producer. The industry processed the canned beans in 2 steps: blanching and canning. A batch of beans were blanched in industry water (soften water) at 85°C for 20 min first, blanching was immediately followed by the canning process as follows: the blanched beans were filled into each can (417g can size) to achieve the equivalent of 81g bean solids in each can. Then the tomato sauce (336g, 325ml) heated up to 91°C by steam injection, and the hot sauce was filled into the can to achieve net weight of 417 g. The whole can was then sterilised in a rotary reel and spiral retort simulator for 7 min at 126°C and 14 min at 128°C (21mins total process time) at 6 rpm.

Canned beans sauce ingredients:

Tomato paste (7%, w/w%), sugar (5%, w/w%), granulated modified starch (1.6%, w/w%), salt (0.9%, w/w%), spirit vinegar (0.8%, w/w%), herbs and spices (0.51%, w/w%) and water (84.2%, w/w%). The sauce was cooked and canned in industry.

2.2 Analysis of mechanical properties

2.2.1 Analysis of firmness using the Texture Analyser

Figure 2.3 shows a picture of TA.XT plus Texture Analyser instrument with a 50 kg load capacity. Four probes were used in this project: 2mm diameter cylinder probe (Figure 2.3 A) was used to assess the hardness of single bean skin; 20mm diameter cylinder probe (Figure 2.3 B) was used to assess the toughness of single half bean cotyledon; Miniature Kramer shear cell and Ottawa cell (Figure 2.3 C, D) were used to assess the toughness of a whole batch of beans (10g), all as described below.

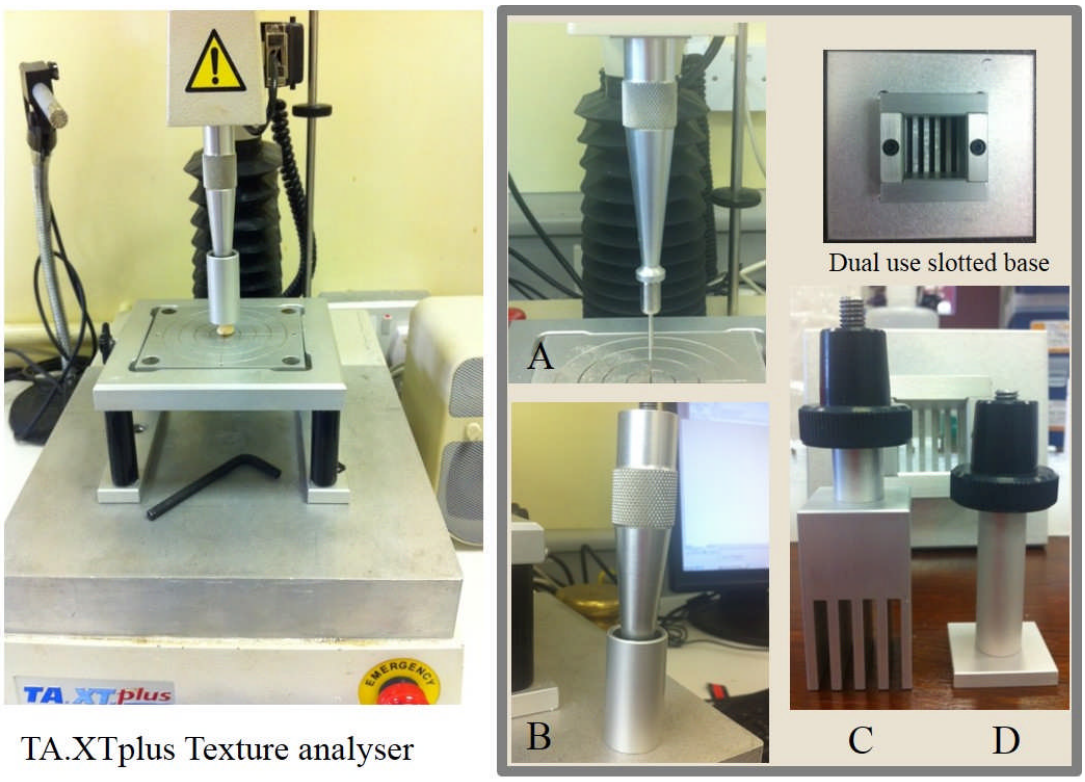

Figure 2.3 TA.XT plus Texture analyser with 50 kg load capacity.

Probes used in this project, 2mm diameter cylinder probe (A), 20mm diameter cylinder probe (B), Miniature Kramer shear cell (C), and Miniature Ottawa cell (D).

2.2.1.1 Puncture test

A 2mm diameter cylinder probe (Figure 2.3 A) was used for the puncture test to assess the hardness of skin tissue. A single bean (with skin) was placed on the platform, it was punctured with the probe at a test speed of 1 $mm*s^{-1}$ to a maximum puncture distance of 3.5 mm. The test speed was adapted from published literature (Revilla and Vivar-Quintana, 2008). All the experiments were done with 10 replicates for each batch of bean.

2.2.1.2 Compression test

A 20mm diameter cylinder probe (Figure 2.3 B) was used for the compression test to assess the toughness of cotyledon. A single half cotyledon (skin removed) was placed on the platform and compressed with the probe at a test speed of 1 $mm*s^{-1}$ to a maximum compression distance of 3.5 mm. The test speed was adapted from published literature (Revilla and Vivar-Quintana, 2008). All the experiments were done with 10 replicates for each batch of bean.

2.2.1.3 Extrusion test

A Miniature Kramer shear cell and Ottawa cell (**Figure 2.3** C and D**)** were used for extrusion test to assess the toughness of batches of beans. A whole batch of beans (10g) without sauce were placed into the dual use slotted base and extruded with either the Miniature Kramer shear cell and Ottawa cell. A Return to Start option was set, with a 2mm*s^{-1} test speed to a maximum extrusion distance of 40mm.

2.2.1.4 Viscosity measurement

A Bohlin C-VOR Shear Rheometer with an axial geometry was used for the measurement of viscosity. One can of beaked beans was opened without shaking in advance. The sauce at the top of the canned beans was removed to a tube using a pipette. The volume of the sauce from each can was recorded. After setting up the rheometer (Bohlin Instruments, High Resolution C – VOR Torque Rebalance) (Figure 2.4), the sauce was poured into the geometry of the rheometer. The viscosity of the samples was measured at a controlled temperature of 25 °C, and procedure was set up as minimum shear rate of 0.1(s^{-1}) and maximum shear rate of 200 (s^{-1}).

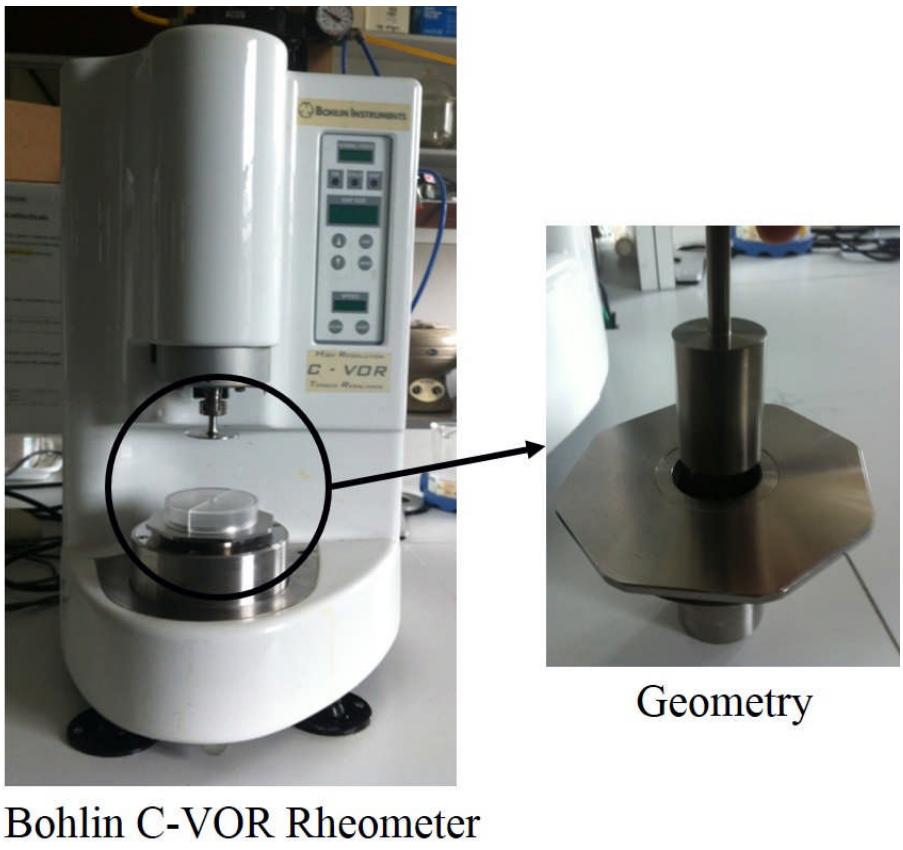

Figure 2.4 Bohlin C-VOR Shear Rheometer and the Geometry

2.3 Monoclonal antibodies

i. JIM5 (Centre for Plant Sciences, University of Leeds).
   Binds to un-methylesterified HG
ii. JIM7 (Centre for Plant Sciences, University of Leeds).
   Binds to methylesterified HG
iii. LM5 (Centre for Plant Sciences, University of Leeds).
   Binds to pectic galactan
iv. LM6 (Centre for Plant Sciences, University of Leeds).
   Binds to pectic arabinan
v. LM1 (Centre for Plant Sciences, University of Leeds).
   Binds to cell wall protein extensin
vi. LM21 (Centre for Plant Sciences, University of Leeds).
   Binds to heteromannan

See Chapter 1, section 1.4.3.3 iii

2.4 Microscopy and immunolocalization

2.4.1 Solutions and reagents

1) 2x PEM buffer, pH=6.9

100mM Pipes (piperazine-N-N'-bis[2-ethane-sulphonic acid]) (Sigma P2949 -100g, UK)

10mM $MgSO_4$

10mM EGTA (ethylene glycol-bis[β-aminoethyl ether-N,N,N',N'-tetraacetic acid]) (Sigma E0396 – 10G, UK)

2) 16% formaldehyde solution (w/v)

16% formaldehyde solution (w/v) methanol – free, 10 x 10 ml ampule (Prod # 28908, Thermo scientific, U.S.A)

3) Fixative medium, pH=6.9

10ml of 16% formaldehyde, 20ml 2x PEM and 10ml dH_2O were mixed and aliquot into 1ml aliquots and freeze. When needed, warm tubes in hand.

4) Wax

90g of Polyethylene glycol 400 distearate (Sigma 305413 – 1KG, UK) was melt with 10g 1-hexadecanol (Sigma 258741 – 500G, UK) in a large beaker in an incubator at 65°C. when melted wax was stirred very thoroughly for several minutes using a stirring bar. The wax was poured into tubes and leave at room temperature to harden. Can be stored at RT and for embedding melt at 37°C.

5) Microtome Blade (10022288, Feather, Japan)
6) Bright Instruments 3500 Microtome
7) Polysine slides (J2800AMNZ, Thermo scientific 25 x 75 x 1.0mm, UK)
8) 0.01% w/v Toluidine Blue O in PBS
   Toluidine Blue O (T3260 – 5G, Sigma, UK)
9) Phosphate-buffered saline solution (PBS)
   Phosphate-buffered saline (PBS) tablets (Fisher chemical BPE9739-1, UK)
10) Bovine serum albumin blocking solution (BSA/PBS)
   3% (w/v) bovine serum albumin in phosphate –buffered saline (BSA; Fisher chemical BPE1605 - 100, UK) in PBS

11) Anti-Rat IgG (whole molecule)-FITC, Developed in Goat, Affinity isolated antigen specific antibody (F6258 – 1ML, Sigma)
12) Calcofluor white stain (18909 – 100ml, Fluka Sigma, Canada)

2.4.2 Preparation of sections for wax-embedded microscopy

The blanched or canned beans cotyledon were cut into 1 cm long cylinders using a scalpel. The beans skins were cut into 1cm square, trying not to squash the sides of the sample. Samples were immediately placed in fixation medium (10ml of 16% formaldehyde, 20ml 2x PEM and 10ml dH_2O) in a 1.5ml eppendorf tube, making sure the bean is in the liquid. For raw beans, beans were placed in fixation medium first and then cut into the size needed. The samples were left overnight in the fixation medium. The fixative was washed away by removing the liquid with a pipette. Firstly, the bean was washed twice with PEM buffer, each time leave it for 20mins. This was following by a wash with PBS buffer twice, each time leave it for 10mins. Then 30% ethanol/water was used to wash two times, each time leave it for 30mins. Then 50% ethanol/water was used to wash two times, each time leave it for 30mins. At last, 70% ethanol was used to wash twice, each time leave it for 30mins. After washing, the further steps should be carried out at 37°C. Samples were moved to 37°C incubator to warm up, then were moved into tubes filled with 1:1 wax:ethanol, and left overnight. 100% wax was added into the samples two times, each time leave for 1hr. Small petri dish and embedding mould were pre-warmed to 37°C, moulds were filled with molten wax, samples were poured into Petri dish and transferred to moulds. Wax was added to moulds so that surfaces were convex. Moulds were left at RT overnight to solidify. The wax samples can be used 12-24hr after embedding and can be stored at RT in a dry place for many years. Sections were cut to a thickness of 10μm using microtome blade on a Bright Instruments 3500 Microtome and collected on polysine-coated slides. Dewaxing and rehydration of the sections was done as by washing with the following solutions: 97% ethanol, 10 mins done three times. 70% ethanol/ water, 10mins, 50% ethanol/ water, 10mins, water, 10mins. PBS buffer, 90mins. After dewaxing, the slides are ready for labelling.

2.4.3 Cytochemical staining

2.4.3.1 Toluidine blue staining of membranes

For preparation of light microscopy, toluidine Blue (0.01% w/v Toluidine Blue O in PBS) was added to the section in order to stain the membranes. Toluidine Blue was incubated for 10 min and was washed out under a gentle stream of tap water and mounted with PBS containing Citifluor AF3 and covered with a glass cover slip, and sections observed under a light microscope (Olympus BH – 2).

2.4.3.2 Preparation for microscopy staining by Calcofluor White

Calcofluor white, which has been show to bind to cellulose micro fibrils (Herth and Schnepf, 1980), was used to localise cellulose.

Sections obtained from the wax-embedded material were incubated with a solution of 25mg/ml calcofluor white (Fluorescent Brightener 28, Sigma, UK) in dH_2O for 30s at RT. Then sections were washed under gentle stream of tap water and mounted with PBS containing Citifluor AF3 and covered with a glass cover slip, and sections observed using a fluorescence microscope fitted with an UV filter (Olympus BH – 2).

2.4.3.3 Preparation for immunofluorescence labelling microscopy

Sections obtained from the wax-embedded material were incubated in Toluidine Blue for 10 min to block the auto-fluorescence. Then Toluidine Blue (0.01% w/v Toluidine Blue O in PBS) was washed out under a gentle stream of tap water. The sections then were incubated in 3% BSA (bovine serum albumin in phosphate – buffered saline) for 1 hour. Sections to be labelled with rat monoclonal antibodies were incubated for 1hr in a solution containing the primary antibody (which in this study were JIM5, JIM7, LM5, LM6, LM1 and LM21) diluted 1:10 in BSA. The sections then washed under a gentle stream of tap water and subsequently incubated for 1hr in a solution containing goat anti-rat IgG linked to fluorescent isothiocyanate (FITC; Sigma, UK) diluted 1:100 in BSA. Then the sections were washed under a gentle stream of tap water and mounted with a glass cover glass slip in PBS containing Citifluor AF3 which is a PBS/ glycerol-based anti-fade solution. Sections were viewed with a microscopy (Olympus BH – 2) equipped with epifluorescence. In control experiment, the primary antibody was omitted.

2.5 Analysis of dietary fibre content

2.5.1 Solutions and reagents

1) Fibre assay kit: K-TDFR 03/2009 (Megazyme international, Bray, Ireland)
   α-amylase (Termamyl, E-BLAAM; 3000 Ceralpha U/ml)
   protease (E-BSPRT; 50 mg/ml)
   amyloglucosidase (E-AMGDF; 3200 U/ml)
2) MES/TRIS buffer, PH-8.2 at 24°C: 0.05M MES, 0.05M TRIS
   MES: 2-(N-Morpholino)ethanesulfonic acid (M-8250, Sigma, UK).
   TRIS: Tris(hydroxymethyl)aminomethane (T-1503, Sigma, UK).
3) 95% Ethanol (459844 Sigma-Aldrich, UK)
4) Miracloth (Calbiochem, La Jolla, CA, USA)

2.5.2 Dietary fibre content by the AOAC method (991.43)

Canned beans samples were analysed for total dietary fibre, water insoluble polysaccharides (WIP) and water soluble polysaccharides (WSP), following an Association of Organic Analytical Chemists (AOAC) (1995) official method (991.43) with minor modifications. It was determined in triplicates with a starting sample weight of 5g.

Beans were homogenized and dissolved in MES/TRIS buffer (pH-8.2) at room temperature, 100ml. Enzyme digestion was performed by incubating the samples with 150 IU of heat stable α-amylase in shaking water bath at 100°C for 35 min. This was followed by incubation with 35 IU of protease for 30 min in shaking water bath at 60°C. Then the pH was adjusted to 4.5 and sample incubated at 60°C with 640 IU amylogucosidase for 30 min in shaking water bath for further starch digestion. Then the digested mixture was precipitated with four volumes of 95% ethanol which had been preheated to 60°C. The mixture was filtered through 3 layers of Miracloth. This is a modification from the original protocol, replacing the sintered glass filter by 3 layers of Miracloth filter. This mode of filtration was found to ease the recovery of the fibre residue without compromising yields (Aldwairji et al., 2014). The residual was washed with ethanol and acetone, then dried in an oven at 103°C until constant weight was achieved. This residue was named water insoluble polysaccharides (WIP). The supernatant was precipitated with four volumes of 95% ethanol and filtered through 3 layers of Miracloth. The residual

obtained from this step was washed with ethanol and acetone, then dried in an oven at 103°C until constant weight was achieved. This residue was named water soluble polysaccharides (WSP). The supernatant obtained from WSP contained the glucose which was derived from starch.

2.6 Analysis of cell wall composition

2.6.1 Solutions and reagents

1) 3% (w/v) Bovine serum albumin blocking solution (BSA/PBS)
   3% (w/v) bovine serum albumin in phosphate –buffered saline (BSA; Fisher chemical BPE1605 - 100, UK) in PBS
2) Tween 20 (Sigma P1379 – 500 ML, UK)
   0.05% Tween in PBS
3) Anti-rat IgG linked to horse-radish peroxidase
   Anti-rat IgG (whole molecule) – peroxidise, Developed in Goat, Affinity isolated antigen specific antibody (Sigma F6258 -1 ML, UK)
4) 3,3′,5,5′-Tetramethyl-benzidine (TMB) Liquid Substrate System for ELISA (Sigma T0440 – 1L, UK)
5) 2 M Sulfuric acid
6) 2 M Trifluoroacetic acid (TFA) (T6508 Sigma-aldrich, UK)

2.6.2 Analysis of extracted polymers using antibody techniques

2.6.2.1 Extraction of polymers

The supernatant of blanched beans was collected to analyse the extracted polymers. The sauce of canned beans was centrifuged using a Beckman Coulter J2 Centrifuge using 250 ml Beckman tubes at 3840 g for 30 min at 20°C. The supernatant was collected to analyse the extracted polymers from canned beans.

2.6.2.2 Enzyme-linked immunosorbent assay (ELISA)

The extracted polymers were determined using ELISA method. The test samples were diluted in 50mM sodium carbonate to PH-9.6. 200µl of solution (antigen) was added to appropriate wells of a sorbent micro titre plate (Thermo Nunc™ MicroWell™ 96-Well Microplates) and incubated overnight at 4°C. Solutions containing antigen were removed. All binding sites on the plate were blocked with 200µl/well 3% (w/v) BSA buffer for 2hr at room temperature. Plates were washed

with PBST (PBS with 0.05% Tween) 3 times. Then to each well was added 100µl/well of the primary antibody (which in this study were JIM5, JIM7, LM5, LM6) diluted 1:10 in BSA buffer for 1.5hr. Then the plates were washed with PSBT 6 times. To the wells were then added 100µl/well of the secondary antibody (anti-rat lgG coupled to horseradish peroxidise (HRP)) diluted 1:1000 in BSA buffer and incubated for 1.5hr. Then the plates were washed with PBST 6 times. Then 150µl/well of HRP substrates TMB was added to each well, and plates incubated for 8-10 mins. At last colour development was stopped by the addition of 35µl/well of 4M H_2SO_4. Absorbance was determined at 450nm in a spectroscopic micro plate reader (Thermo-MUITISKAN FC). For the control sample, the primary antibody was omitted. Three replicates were applied for individual samples.

2.6.3 Analysis of cell wall monosaccharide composition

2.6.3.1 Hydrolysis of cell wall polysaccharides

0.016±0.001g of water insoluble polysaccharides (WIP) and water soluble polysaccharides (WSP) were hydrolysed in 2 M TFA for 1h at 120°C. TFA was removed by evaporation under vacuum, adding water and repeating for the evaporation 6 times. The hydrolysates were reconstituted in ultra-filtered deionized water containing 0.1µg/10µl internal standard fucose, filtered through 0.45 um PTFE filters, and placed in injection vials (200 µl volume) with slotted lids.

2.6.3.2 Analysis of monosaccharides by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

Monosaccharide content and composition was determined using HPAEC-PAD. Figure 2.5 shows the picture of the instrument, a Thermo Scientific Dionex ICS-5000 system used in this study.

HPAEC-PAD has been found to have better recovery and is a sensitive method to determine the complex carbohydrate resulting from the hydrolysis of non-starch polysaccharides (Henshall, 1999). Pulsed Amperometric Detection (PAD) provided more specificity and more compatibility with gradient elution than RI (refractive index) in HPLC. Therefore, HPAEC-PAD was believed to be a good method to determine the cell wall composition for this project.

Figure 2.5 Thermo Scientific Dionex ICS-5000 system

Cell wall monosaccharide was determined by HPAEC-PAD of hydrolysed material. A PA20 column (Dionex, USA) was used at a flow rate of 0.5 ml/min. Before the injection of each sample, the column was washed with 200 mM NaOH for 10 min, then equilibrated with 10 mM NaOH for 10 min.

The elution program consisted of an isocratic elution with 10 mM NaOH from 0 to 37 min, followed by a linear gradient up to 800 mM NaOH from 37 to 43 min, and finally down to 10mM NaOH from 43 to 45 min. Monosaccharide standards included L-Fuc, L–Rha, L-Ara, D-Gal, D-Glc, D-Xyl, D-Man, D-GalUA and D-GlcA (Sigma, UK), the standard mixture concentration range from 0.001µg/10µl to 0.1 µg/10µl. A standard mixture run was performed before analysis of a batch of samples for verification of the response factors.

2.6.3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test (Shrivastava and Gupta, 2011). Formula LOD=3SD/b and LOQ=10SD/b (SD is the standard deviation of the response and b is the slope of the regression line) were

used to calculate LOD and LOQ (Shrivastava and Gupta, 2011). Table 2.2 shows the LOD and LOQ of different monosaccharides, determined experimentally.

Table 2.2. LOD and LOQ of different monosaccharides.

Monosaccharides	LOD(µg/µl)	LOQ(µg/µl)
Fucose	2.82×10^{-4}	9.40×10^{-4}
Rhamnose	3.91×10^{-4}	1.30×10^{-3}
Arabinose	2.04×10^{-4}	6.80×10^{-4}
Galactose	2.69×10^{-4}	8.96×10^{-4}
Glucose	1.09×10^{-4}	3.62×10^{-4}
Xylose & Mannose	1.84×10^{-4}	6.12×10^{-4}
galacturonic acid	6.54×10^{-4}	2.18×10^{-3}
glucuronic acid	7.34×10^{-4}	2.45×10^{-3}
raffinose	5.95×10^{-4}	5.51×10^{-4}
stachyose	1.10×10^{-3}	1.02×10^{-3}

2.7 Analysis of starch and extracted oligosaccharides

2.7.1 Solutions and reagents

1) Fibre assay kit: K-TDFR 03/2009 (Megazyme international, Bray, Ireland)
   α-amylase (Termamyl, E-BLAAM; 3000 Ceralpha U/ml)
   protease (E-BSPRT; 50 mg/ml)
   amyloglucosidase (E-AMGDF; 3200 U/ml)
2) MES/TRIS buffer, PH 8.2 at 24°C: 0.05M MES, 0.05M TRIS
   MES: 2-(N-Morpholino)ethanesulfonic acid (M-8250, Sigma, UK).
   TRIS: Tris(hydroxymethyl)aminomethane (T-1503, Sigma, UK).
3) 95% Ethanol (459844 Sigma-Aldrich, UK)
4) Miracloth (Calbiochem, La Jolla, CA, USA)
5) 3,5-Dinitrosalicylic acid (DNS) (128848 Sigma Aldrich, UK)

2.7.2 Analysis of starch content

2.7.2.1 Digestion of starch

5g of beans were homogenized and dissolved in MES/TRIS buffer (PH-8.2) at 24°C, 100ml. Starch was hydrolysed by enzyme digestion. Firstly, samples were incubating with 150 IU of heat stable α-amylase in shaking water bath at 100°C for 35 min. Secondly, samples were incubating with 35 IU of protease for 30 min in shaking water bath at 60°C. At last, adjust the PH to 4.5 with HCl and incubated at 60°C with 640 IU amylogucosidase for 30 min in shaking water bath for further starch digestion. After filtration through miracloth, the supernatant was collected for glucose analysis by HPAEC-PAD or DNS

2.7.2.2 HPAEC-PAD

The glucose which was derived from starch was determined using HPAEC-PAD. A PA20 column (Dionex, USA) was used on chromatography at a flow rate of 0.4 ml/min. Before the injection of each sample (up to 0.1 μg glucose), the column was washed with 200 mM NaOH for 10 min, then equilibrated with 60 mM NaOH for 10 min.

The elution program consisted of an isocratic elution with 60 mM NaOH from 0 to 15 min, followed by a linear gradient up to 200 mM NaOH from 15 to 20 min, and finally down to 60mM NaOH from 20 to 25 min. Glucose standard and internal standard fucose were from Sigma, UK.

2.7.2.3 3,5-Dinitrosalicylic acid assay (DNS)

The reducing sugar (include glucose which was derived from starch) was determined using DNS assay. 1ml of sample was pipette into a test tube. Then 1ml of DNS regent and 2ml of distilled water were added to the tubes (total volume = 4ml). The tubes were placed in a boiling water bath (100°C) for 5min to allow the reducing sugar (include glucose) to react with the DNS. After that, the solution was allowed to cool down. Then 16ml of water was pipetted into the tube and mixed. After the reaction stopped, the absorbance was read in the spectrophotometer (Aquarius - CECIL 7200) at 540nm.

2.7.3 Analysis of extracted oligosaccharides

2.7.3.1 Extraction of oligosaccharides

Raw beans were ground into powder by blender. 1g of bean powder was incubated in 30ml 70% ethanol for 30min at 43°C. Then the sample was centrifuged at a speed of 3000rmp for 15min. After that the sample was filtered with filter papers (Whatman No. 1). The supernatant was collected to analyse the oligosaccharides which have been extracted.

2.7.3.2 HPAEC-PAD

The extracted oligosaccharides (e.g. raffinose and stachyose) were analysed using HPAEC-PAD. The elution program was used same as the glucose elution program (section 2.7.2.2).

2.8 Statistical methods

Figure 2. 6 shows the flow chart of statistical analysis. In order to test the effect of cultivar and region, data needed to be checked the normality assumption first before applying the ANOVA. Shapiro-Wllk test was used to check the normality. If the data was normal distribution, a two-factor ANOVA was applied. If the data was not normal distribution, the data needed to be transformed to logarithm or square root of original data form. After transformation, check the normality assumption again, if the data was normal distribution, a two-factor ANOVA can be applied with the transformation data. If the data was still not normal distribution after transformation, a non-parametric ANOVA Kruskal Wallis was applied. When two-factor ANOVA applied, interactions were checked first. If the interaction was significant between cultivar and region. Multiple comparison Tukey's HSD test was applied to determine which groups in the same differ significant. If the interaction is not significant between cultivar and region, one-factor ANOVA was applied for cultivar and region separately. For cultivar or region one-factor ANOVA, when the differences were significant, multiple comparison Tukey's test on was applied to determine which groups in the same differ significant.

To test the correlation between different properties, Pearson's correlation test were applied.

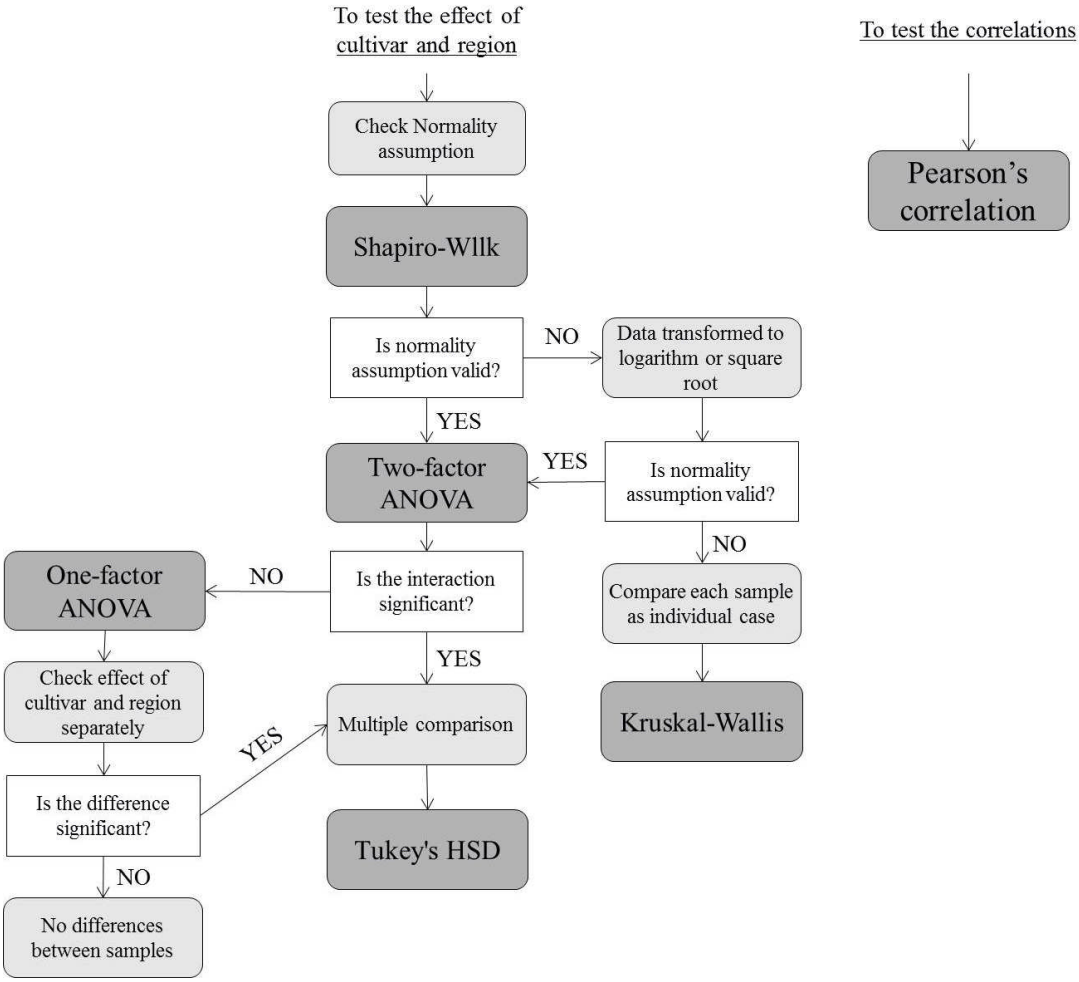

Figure 2. 6 Flow chart of statistical analysis

2.8.1 Two – factor ANOVA

Two-factor analysis of variance is an extension to the one-factor analysis of variance. It examines the influence of two different categorical independent variables. The two-factor ANOVA would determine the main effect of contribution of each independent variable as well as the interaction effect between the independent variables. The assumptions for two-factor ANOVA are: 1) The populations from which the samples were obtained must be normally distributed. 2) The samples must be independent. 3) The variances among the populations must be equal. 4) The groups must have the same sample size. In this project, statistical differences were considered at a 0.05 level of significance (Valen, 1970). Therefore, in order to meet the assumption for the two-factor ANOVA, eight types of beans from 2 cultivars and 4 regions were used to investigate the effect of cultivar and

region on the properties of canned beans. In this project, ANOVA was done using a 90% or 95% confidence interval.

2.8.2 Tukey's HSD (honest significant difference) test

Tukey's HSD test is a multiple comparison test. When an analysis of variance (Two-factor ANOVA) gave a significant result, Tukey's HSD was performed to determine which groups in the same differ significantly. In this project, multiple comparisons of treatment means was done using a 90% or 95% confidence interval based on Tukey’s honestly significant difference (HSD).

2.8.3 Kruskal Wallis test

Kruskal Wallis non-parametric ANOVA is applied when the data is not normal distribution. Kruskal-Wallis compares between the medians of two or more samples to determine if the samples have come from different populations. The data must be independent from each other, but they do not have to be equal. Kruskal Wallis test was done using a 95% confidence interval, each samples were treated as individual samples.

2.8.4 Pearson correlation

Pearson correlation is used to measure the correlations coefficient in this project. Linear correlation significant level was measured using SPSS. Pearson correlation was done using a 95% confidence interval.

2.8.5 Statistic tools

Software R (The R Project for Statistical Computing) and software SPSS Statistic were used in this project for the statistical analysis.

Chapter 3

Physical and mechanical properties of canned baked beans

3.1 Introduction

Texture is an important quality attribute in cooked food, and it is related to physical and mechanical properties of the material, including properties such as water imbibition (water content) and firmness (Anzaldua-Morales and Brennan, 1982). From the observation by Heinz industry, under the same processing procedure, beans from different cultivar and growth region gave inconsistent textural quality of the canned baked beans. Also, importantly, the physical property – water absorption capacity would significantly affect the commercial benefit for the industry (higher water absorption of the beans would decrease the cost). Therefore, in this chapter, the physical and mechanical properties of beans of different cultivars from different growth regions was investigated after each processing step (including blanching and canning).

In this chapter, the aims were to: 1) to characterise the beans in terms of physical properties (size, weight, water capacity/drained weight); 2) to develop suitable methodology for analysis of mechanical properties of beans; 3) to apply this methodology to investigate the effect of growth region and cultivar on physical and mechanical properties; 4) to investigate the effect of processing on mechanical properties.

The physical and mechanical properties of seventeen bean samples were studied initially. Samples were from 9 cultivars grown in 5 regions in the United States, Canada and China (chapter 2, section 2.1) provided by a commercial producer. For the sake of statistical balance, 8 types of beans were selected to study the effect of cultivar and region.

Beans were investigated after two different thermal processes, which were blanching and canning (processing flow chart is shown in Figure 3.1), the mechanical properties of the beans were determined using TA.XT plus Texture Analyser to assess the firmness of the blanched and canned beans, different probes were used to assess the different sections of the beans including skin, cotyledon and the whole

batch of beans. The mechanical properties of the sauce were investigated using a Bohlin Rheometer.

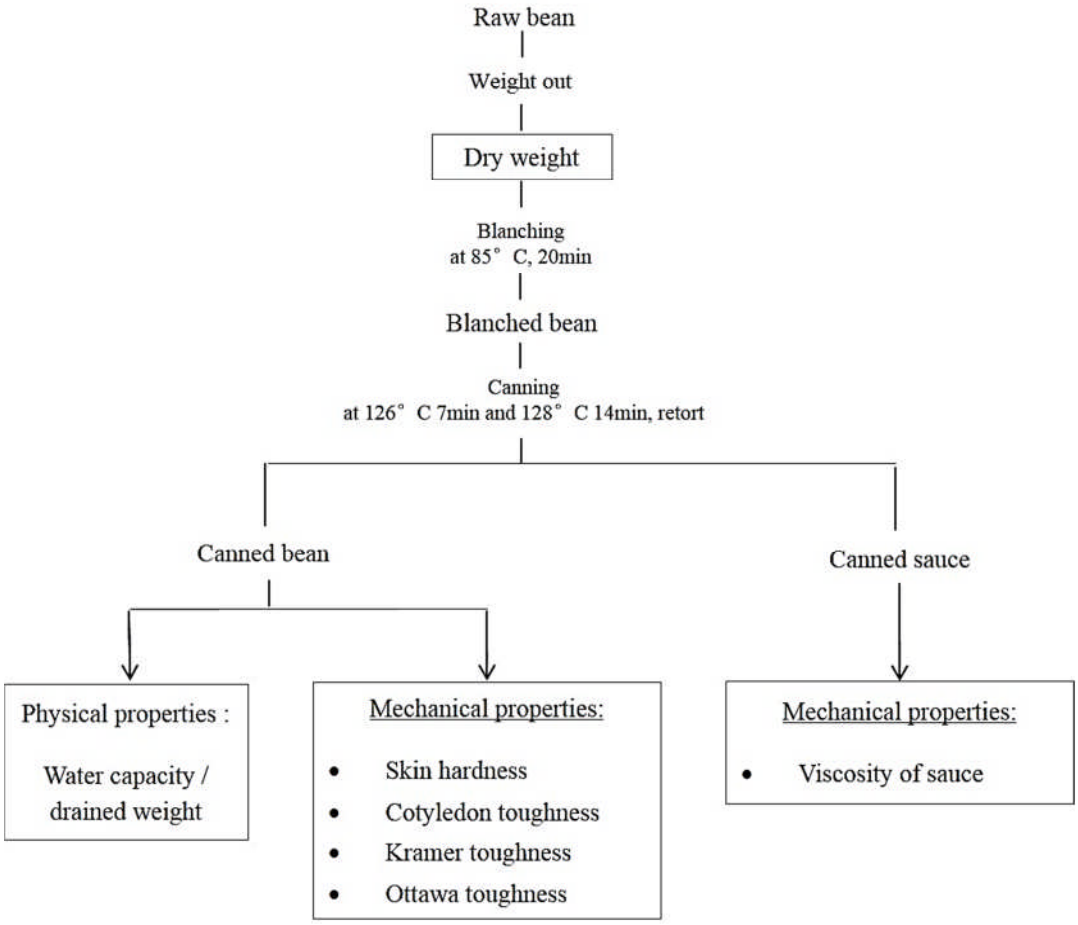

Figure 3.1 General flow chart of the processing procedure of canned beans, and the measurement of canned beans and canned sauce.

3.2 Physical characteristics of raw, blanched and cooked beans

Seventeen types of beans were initially analysed which were from nine cultivars grown in five different regions. The large number of cultivars compared to the number of growing regions made the results statistically unbalanced with unequal samples for each stratification. Thus, the sample size was reduced to eight samples from two cultivars grown in four different regions which met the assumptions for the two – factor ANOVA (the variances of the populations must be equal and the groups must have same sample size (Chapter 2, section 2.8.1). Therefore, further analyses were focused on the eight types of samples.

3.2.1 Effect of cultivar and region on the physical characteristics for raw beans

The raw beans' radial thicknesses ranged from 3mm to 4mm, they were ellipsoid or round in shape, and off white in colour. The dry bean weight ranged from 0.2 g to 0.3 g (Table 3.1). Numerically, bean C1 which had the biggest dry weight, it was 57.6% (w/w %) heavier than Ch09 which had the smallest dry weight. Chinese beans harvested from different years present differently, the average weight for Ch10 was higher than Ch09 (by 22.8%, w/w %).

Table 3.1 Dry bean weight (g) of seventeen types of beans.

	Raw bean weight (g)
A1	0.231±0.012
B1	0.232±0.009
C1	0.290±0.012
D1	0.228±0.01
A2	0.229±0.006
B2	0.233±0.01
C2	0.239±0.008
D2	0.218±0.013
B3	0.206±0.008
D6	0.209±0.01
D7	0.236±0.009
C5	0.248±0.01
C4	0.224±0.011
E5	0.226±0.008
F8	0.252±0.01
Ch09	0.184±0.01
Ch10	0.226±0.009

Values show mean of 20 replicates plus and minus the standard error of mean at 95% confident interval.

In order to investigate the effect of cultivar and region on the dry bean weight, a two-factor ANOVA needed to be carried out. The assumptions of ANOVA and Tukey test are: the variances among the populations must be equal; the groups must have the same sample size. Thus 8 types of beans from 2 cultivars and 4 regions were selected to investigate the effect of cultivar and region. The statistical analysis shows that cultivar and region had a significant effect at a 99.9% confidence

interval, and their interaction was also significant (Table 3.2). Bean C1 had significant highest dry weight than all the other beans (21.3% - 33%, w/w %), so that C1 may be the main reason causing the significant differences. Therefore, region C was removed and the analysis recalculated. Table 3.2 shows that after removing beans from C, none of the factors are significant. Therefore, cultivar and region was not found to have significant effect on the dry bean weight excluding the special heavy beans from C. Except the beans from region C are heavier, no differences were found for beans from region A, B and D.

Table 3.2 Two - factor ANOVA P-value for dry bean weight of eight types of beans / eight types exclude C.

	Dry weight	Dry weight without C
Cultivar	<0.001	0.35
Region	<0.001	0.12
Cultivar : Region	<0.001	0.52

Analysed after data transformation (log of original data) at 95% confidence level.

3.2.2 Effect of cultivar and region on the physical characteristics for blanched beans

After blanching, the radial thickness of the beans increased from 3mm – 4mm to 6mm – 7mm for all beans. Beans absorbed 0.15 g – 0.2 g of water and the water capacity was absorbing 0.6 g – 0.8 g of water per gram of beans.

Table 3.3 shows the water capacity of all the beans, which is described as the absorbed water per gram of beans after blanching. Numerically C1 which had the smallest water capacity absorbed 20% less water than E5 which had the largest water capacity.

In order to investigate the effect of cultivar and region on the water capacity, two-factor ANOVA was carried out for eight types of beans. Table 3.4 shows that only region affect the water capacity of the blanched beans significantly in a 99.9% confident level. Beans from C had significant smallest water capacity (Figure 3.2), region C had 8% - 13% less water capacity than A, B and D.

Quenzer et al. (1978) studied the water imbibition during soaking of pinto beans, they claimed that the location significantly affected the water imbibition, beans from El Paso had less water imbibition compared to the other two locations. Another study reported about the effect of blanching temperature on the drained weight of navy bean, black bean and pinto bean (Balasubramanian et al., 2000), however, no studies were found to investigate the effect of region and cultivar to water capacity for blanched navy beans. Region significantly influenced the blanched navy beans water capacity. Therefore, growth region was found have significantly effect on the pre-cooking beans water capacity, and possibly affect the water capacity of canned beans consequently.

After blanching, the navy beans remained intact, cotyledon and skin were not splitting or cracking, different blanching methods were found not affect degree of splitting in the final product (Nordstrom and Sistrunk, 1979). And beans still had a hard texture which was not suitable for eating.

Table 3.3 Water capacity of blanched beans.

	Water capacity (g/g bean)
A1	0.798±0.041
B1	0.73±0.044
C1	0.662±0.063
D1	0.774±0.049
A2	0.786±0.044
B2	0.767±0.048
C2	0.698±0.037
D2	0.754±0.031
B3	0.689±0.043
D6	0.777±0.029
D7	0.787±0.03
C5	0.767±0.029
C4	0.777±0.027
E5	0.829±0.02
F8	0.746±0.032
Ch09	0.775±0.019
Ch10	0.728±0.051

Values show mean of 10 replicates plus and minus the standard error of mean at 95% confidence interval.

Table 3.4 Two - factor ANOVA P-value for water capacity of eight types of blanched beans.

	Water capacity
Cultivar	0.63
Region	<0.001
Cultivar : Region	0.38

Analysed after data transformation (Square root of original data) at 95% confidence interval.

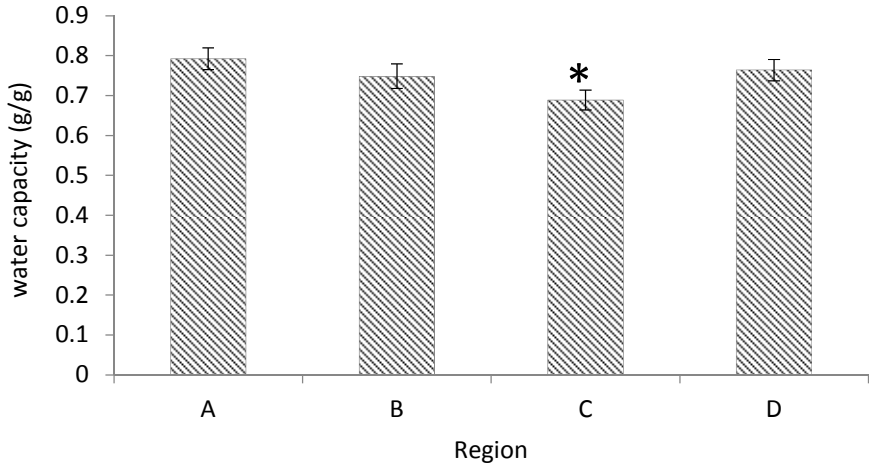

Figure 3.2 Water capacity of eight types of blanched beans stratified by region.

Values show mean of 10 replicates +/- standard error of mean at 95% confident interval. * Means significantly different at p<0.05 levels.

3.2.3 Effect of cultivar and region on the physical characteristics of canned beans

Beans were canned in a retort in commercial producer at 126°C for 7 min and then 128 °C 14min after blanching. After canning, the single wet bean without sauce weighted ranged from 0.4 g to 0.5 g, bean absorbed 0.25 g – 0.33 g of water and the water capacity after canning ranged from 0.5 g – 1.9 g of water per gram of beans. The water capacity was calculated according to the formula shown below:

$$water\ capacity\ (\frac{g}{g}) = \frac{single\ wet\ weight\ (g) - single\ dry\ weight(g)}{single\ dry\ weight\ (g)}$$

Washed drained weight is described as the weight of canned beans without the sauce. The drained weight was calculated from single drained weight. Dry bean weight in one can is 81g, so that the formula is shown below:

$$drained\ weight\ (g/can) = \frac{single\ wet\ weight\ (g) \times 81\ (g)}{single\ dry\ weight\ (g)}$$

As the formulas for water capacity and drained weight are highly correlated, drained weight was chosen for analysis and to associate with other characteristics for canned beans for the sake of industry measurement convenience (no need to weight out every single bean water capacity).

Figure 3.3 shows the drained weight (g/can) for eight types of canned beans. C1 and C2 had significantly lower drained weight than A1 and D2 (average 7%, w/w %). To investigate the effect of region and cultivar on the drained weight of canned beans, two-factor ANOVA was carried out (Table 3.5). Interactions were found between cultivar and region, cultivar and region both had significant effect on drained weight and region affect drained weight more than cultivar. The interaction between cultivar and region may be caused by beans from D. In region D, cultivar 2 had higher drained weight than cultivar 1, however beans from the other regions behaved contrarily. Beans from region C had significant higher drained weight than beans from region A.

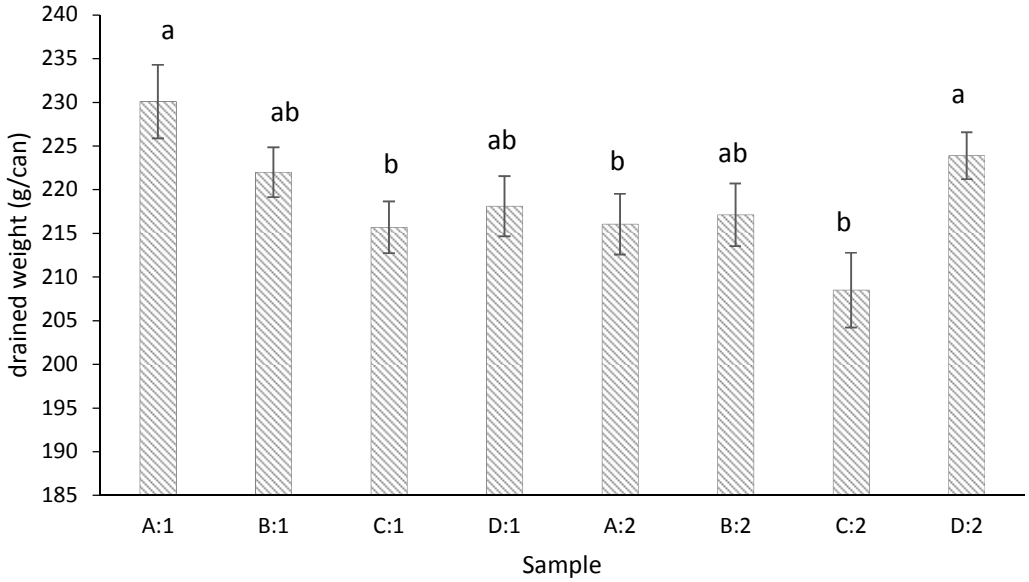

Figure 3.3 Drained weight (g/can) for eight types of canned beans.

Values show mean of 3 replicates +/- standard error of means at 95% confident interval. Letters a, b, c means within same column with the same superscript letters are not significantly different at p<0.05 levels.

Table 3.5 Two factor ANOVA P-value of drained weight for eight types of canned beans.

	Drained weight
Cultivar	0.023
Region	0.0042
Cultivar : Region	0.019

At 95% confidence interval

Significant differences in cultivars were noted for drained weight of beans canned in brine (Wang and Chang, 1988). Wang et al. indicated that differences in drained weight of navy beans canned in brine were found among different cultivars which were grown in one region, Upland and Fleetwood had higher drained weight than C-20 or Seafarer beans (Wang et al., 1988). However, it is not possible to comparable these projects because different cooking medium may have different effect on the final product. Also, the differences may be cultivar dependent. For the samples used

in this project, both cultivar and region were found to significantly affect the drained weight of canned beans with interactions.

Beans with higher water capacity gain final weight, lowering the initial dry bean weight require in the can, consequently benefit the industry by reducing the cost. Drained weight was found to correlate with other properties, for examples, negative correlation was found between drained weight and visual appeal (r= - 0.26 to -0.66) (Walters et al., 1997). Drained weight was also associated negatively with firmness of navy beans (Lu and Chang, 1996, Wang et al., 1988, Balasubramanian et al., 2000). Besides, drained weight is very easy and convenience to obtain. Therefore, the whole can beans drained weight may be a good prediction parameter to predict beans other physical and chemical properties. The correlations between drained weight and other properties in this project will be discussed in chapter 7.

After canning, the beans were very soft, skins were splitting or cracking a little bit, but two cotyledons remained together, bean texture became soft and suitable for eating. Although splitting was not discussed in this project, it is suggested that high splitting and clumping would decrease the canning quality and overall acceptance (Lu and Chang, 1996).

3.3 Mechanical properties of single beans.

The mechanical properties of raw beans were not analysed because the limitation of the equipment, the TA.XT Texture Analyser had a 50 kg load cell that did not produce sufficient force to break the tissue. The raw beans require more than 100N forces to break (Jokanović et al., 2012). Therefore, blanched and canned beans mechanical properties were measured.

3.3.1 Skin hardness of single beans

To assess the skin hardness of individual beans, a penetration measurement is needed. The probe needs to be smaller than the sample's surface. Two kinds of probes met the requirement, the needle probe and 2mm diameter cylinder probe. Needle probe has an extremely small contact area so it needed a very small force to puncture through the skin, around 0.6 N for navy bean skin (data not shown). The small force obtained from needle probe detected small difference because of the limitation of detection (data not shown). Therefore, a 2mm diameter cylinder probe

was selected to assess the navy bean skin hardness. The probe penetrated the surface of a single bean at a speed of 1 mm**s^{-1} (Chapter 2. Section 2.2.1.1), the speed was chosen to correspond with that used by Revilla (Revilla and Vivar-Quintana, 2008). A previous study indicated that speed did influence the results but not to the extent that variations between different samples could not be detected (Anzaldua-Morales and Brennan, 1982).

The probe puncture through the skin of the beans to obtain a puncture curve, it represented as Type C curve in Figure 1.4 with a negative slop mentioned in Chapter 1. A typical force-distance curve obtained from such apparatus is shown in Figure 3.4. At point A, the probe made contact with the bean's surface and pressure was applied on the beans, an initial rapid rise in force is observed. Then the bean was compressed by the probe and the bean deformed under the pressure, but no rupture or breaking happened from point A to point B. This stage ends abruptly when the probe punctures through the skin and begins to penetrate into the bean cotyledon, this event is represented by the sudden change in slope called the 'yield point' (point B). The skin tissue stretched until point B and reached the maximum force that the skin can resist, the skin ruptured and the probe penetrated through the skin onto the cotyledon tissue below. The force required to penetrate the cotyledon after is lower. From point B to C, the probe went through the cotyledon which is much softer than skin. The yield point B represented the force needed to penetrate the skin which is defined here as skin hardness in this thesis. As the radial thickness of the bean was 6mm – 7mm, the probe has to go at least half through the bean to make sure the skin was punctured through, so that 3.5 mm was chosen to be the distance that probe penetrated.

Revilla and Vivar-Quintana (2008) had used 2mm diameter cylinder recording peak of maximum force to measure the Faba beans skin firmness.

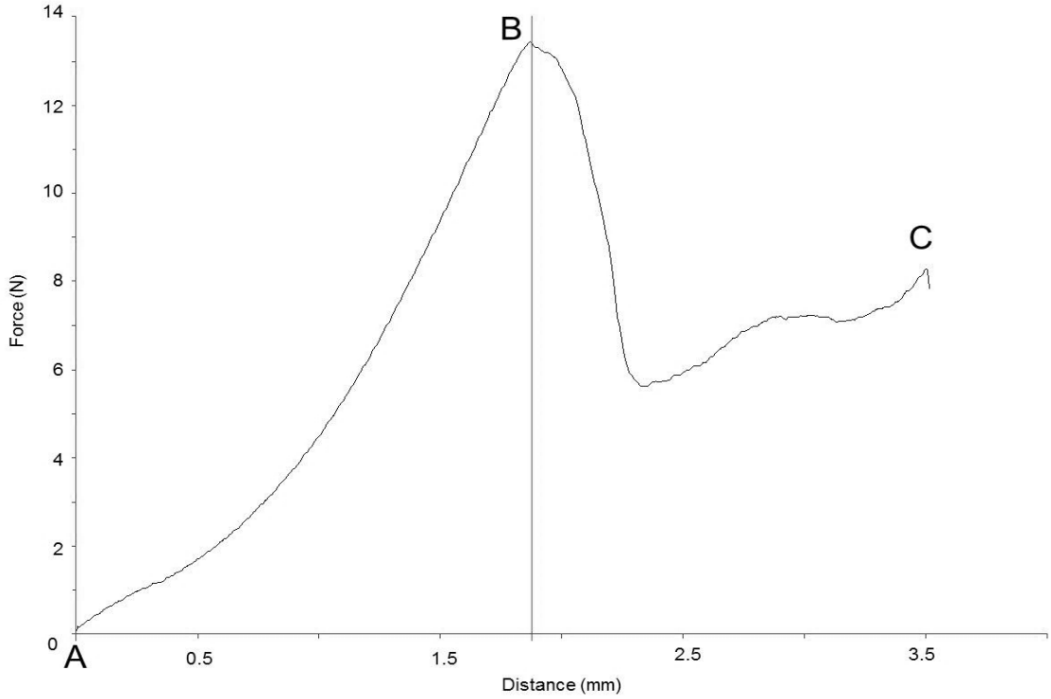

Figure 3.4 Respresentative diagram of force versus distance produced by the penetration of skin tissue using 2mm diameter cylinder probe at speed 1mm*s^{-1}.

3.3.2 Cotyledon toughness of single bean

To assess the cotyledon toughness of individual beans, a compression measurement is needed. In this project, a 20mm diameter cylinder was used to analyse the toughness of the cotyledon (without skin) at a speed of 1 mm*s^{-1} (Chapter 2, section 2.2.1.2), same speed as skin hardness procedure, the speed was chosen to correspond with that used by Revilla (Revilla and Vivar-Quintana, 2008). Because the two cotyledons were very easy to split under the pressure and interrupted the results, only one cotyledon was analysed (half bean) to increase consistency. The force-distance curve obtained is shown in Figure 3.5. A half single bean (one cotyledon) was compressed under the probe, at point A, the probe made contact with the beans, the force increased as the cotyledon deformed under the pressure of the probe. At point B, the outer layer of the cotyledon (epidermal layer) ruptured so that the force decreased after this point. From point B to point C, compression of the remaining of the cotyledon continued and the force kept increasing till the procedure finished. From point A to B the graphs represents the force needed to rupture the outer cellular layers of the cotyledon, and from B to C represents the force needed to

continue compress the bean. The positive area from A to C represents the work needed to complete the compression to the bean and is defined as the toughness of the cotyledons in this thesis. As the radial thickness of the bean was 6mm – 7mm, the probe has to go at least half through the bean to crush the cotyledon, so that 3.5 mm was chosen to be the distance that probe compressed down.

Bay et al. (1996) used a TA.XT with a 37mm diameter cylinder compression probe to measure whole snap bean and 1mm diameter cylinder probe was used to analyse the cotyledon of the snap bean (Bay et al., 1996). Revilla and Vivar-Quintana (2008) used 10mm diameter cylinder probe to record peak force to measure the Faba beans cotyledon section.

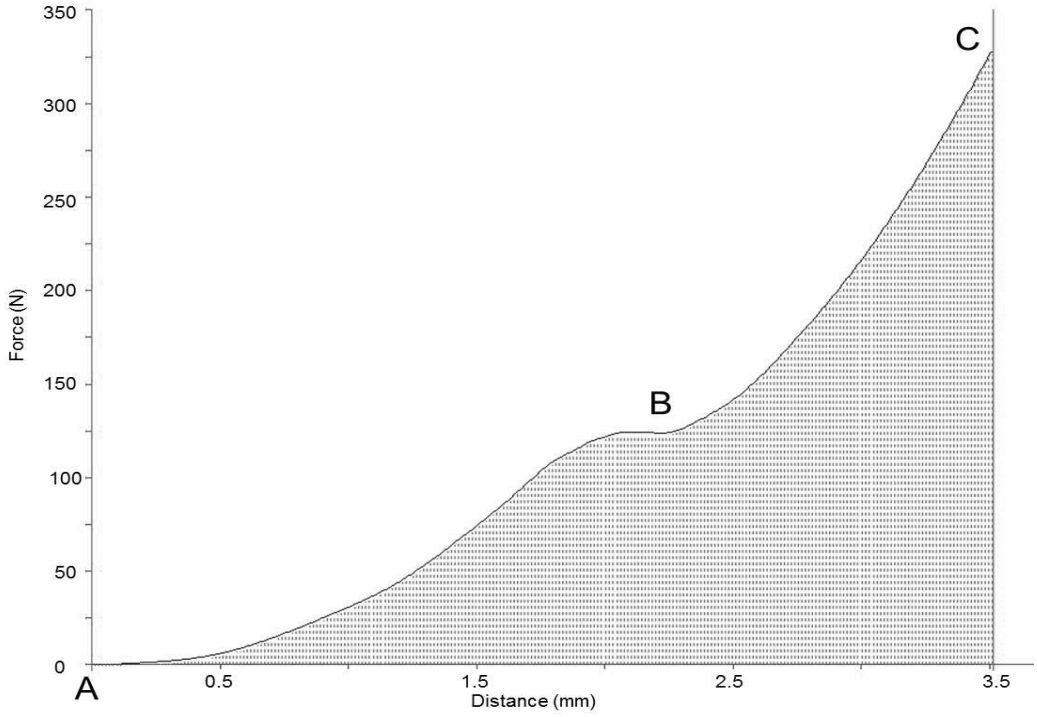

Figure 3.5 Respresentative diagram of force versus distance produced by the compression of cotyledon tissue using 20mm diameter cylinder probe at speed 1mm*s^{-1}.

Table 3.6 shows skin hardness and cotyledon toughness for all types of blanched beans. E5 and F8 had relatively softest skin, Ch09 and A2 had relatively hardest skin. C4 and C5 had relatively softest cotyledon, B2 and C2 had relatively toughest cotyledon. From the table, no obvious correlation was seen between skin and cotyledon, hard skin was not necessarily associated with having a tough cotyledon. Not all types of canned beans were analysed because some of them were not

available. Only eight types of beans were analysed, which is shown in the next section.

Table 3.6 Skin hardness and cotyledon toughness for all types of blanched beans.

	Skin hardness (N)	Cotyledon toughness (N*mm)
A1	0.638±0.083	56.4056±14.3
B1	0.716±0.061	64.9928±11.2
C1	0.705±0.088	55.2492±15.8
D1	0.661±0.065	49.4736±7.1
A2	0.732±0.067	59.614±15.8
B2	0.691±0.066	70.8078±9.2
C2	0.781±0.07	66.3884±15
D2	0.696±0.082	60.8909±16.2
B3	0.709±0.067	56.375±13.5
D6	0.709±0.054	53.3106±18.5
D7	0.654±0.062	54.5468±23.3
C5	0.67±0.043	45.4325±5.6
C4	0.668±0.062	50.6255±13.7
E5	0.605±0.036	52.5704±8.7
F8	0.604±0.094	62.1386±15.2
Ch09	0.743±0.059	63.4241±13
Ch10	0.67±0.06	63.4892±14.8

Values show mean of 10 replicates plus and minus the standard error of mean at 95% confidence interval.

3.3.3 Effect of cultivar and growth region on the mechanical properties of single bean for eight types of beans.

Table 3.7 shows the skin hardness and cotyledon toughness of blanched beans and canned beans. In order to investigate the effect of cultivar and region effect on mechanical properties of single bean, two-factor ANOVA was carried out, as shown in Table 3.8.

For blanched beans, numerically D2 which had the hardest skin was 23% harder than A1 which had the weakest skin. B2 which had the toughest cotyledon was 43% tougher than D1 which had the softest cotyledon, however there is no statistically significant differences amongst bean cotyledons because of the big variance of the data (30% variation, ratio of the standard deviation to the mean), big variance was caused by biological variations that more cells were compressed when measured the cotyledon than the skins, because bigger arear was compressed for cotyledon than skin. Table 3.7 shows that for blanched beans, only skin hardness was affected by cultivars, beans from cultivar 2 had harder skin than cultivar 1. Cultivar and region were not found to have significant effects on the single bean cotyledon toughness for blanched beans.

For canned beans, numerically C2 which had the hardest skin and was 37% harder than D1 which had the weakest skin. C2 which had the toughest cotyledon was 53% tougher than D2 which had the softest cotyledon. Table 3.8 shows that region had significant effect on both skin hardness and cotyledon toughness after canning, interaction happened between cultivar and region for cotyledon toughness. Beans from C had harder skin and tougher cotyledon than beans from A and D. Interaction was observed for cotyledon toughness. Beans from cultivar 2 were tougher than cultivar 1 in regions A and C, but is regions B and D they performed in an opposite way.

The canning process dramatically changed the mechanical properties of single beans. Skin hardness decreased dramatically after canning from 15N to 1N. Besides, the distance from deformation to the first skin breakage point (Figure 3.4, x-axis of point A to B) decreased significantly from 2.5mm to 1.5mm (Figure 3.6). Hence not only the hardness but also the deformability of the skin reduced after canning process. Cotyledon toughness decreased dramatically compared to blanched beans cotyledon by 83% - 89%. Therefore, the canning processing softened both the skin

and cotyledon texture considerably. However, the softening did not lead to disintegration of the bean structure.

Table 3.7 Skin hardness and cotyledon toughness of blanched beans and canned beans for eight types of beans.

	Blanched beans		Canned beans	
Bean name	Skin hardness	Cotyledon toughness	Skin hardness	Cotyledon toughness
A1	14.428[a]	56.406	0.877[ab]	7.447[a]
B1	14.599[a]	65.000	0.955[bc]	8.605[ab]
C1	15.345[a]	55.249	1.045[c]	9.467[bc]
D1	15.204[a]	49.474	0.782[a]	7.946[ab]
A2	15.993[b]	59.614	0.928[ab]	8.404[a]
B2	15.946[b]	70.808	0.970[bc]	8.115[ab]
C2	16.675[b]	66.388	1.072[c]	11.091[c]
D2	17.740[b]	60.891	0.846[a]	7.252[a]

Values show mean of 10 replicates. Letters a, b means within column with the same superscript letters are not significantly different at $p<0.05$ levels.

Table 3.8 Two factor ANOVA P-value for skin hardness and cotyledon toughness of blanched beans for eight types of beans.

	Blanched beans		Canned beans	
	Skin hardness	Cotyledon toughness	Skin hardness	Cotyledon toughness
Cultivar	<0.001	0.065	0.14	0.37
Region	0.092	0.18	<0.001	<0.001
Cultivar : Region	0.60	0.87	0.91	0.011

At 95% confidence interval.

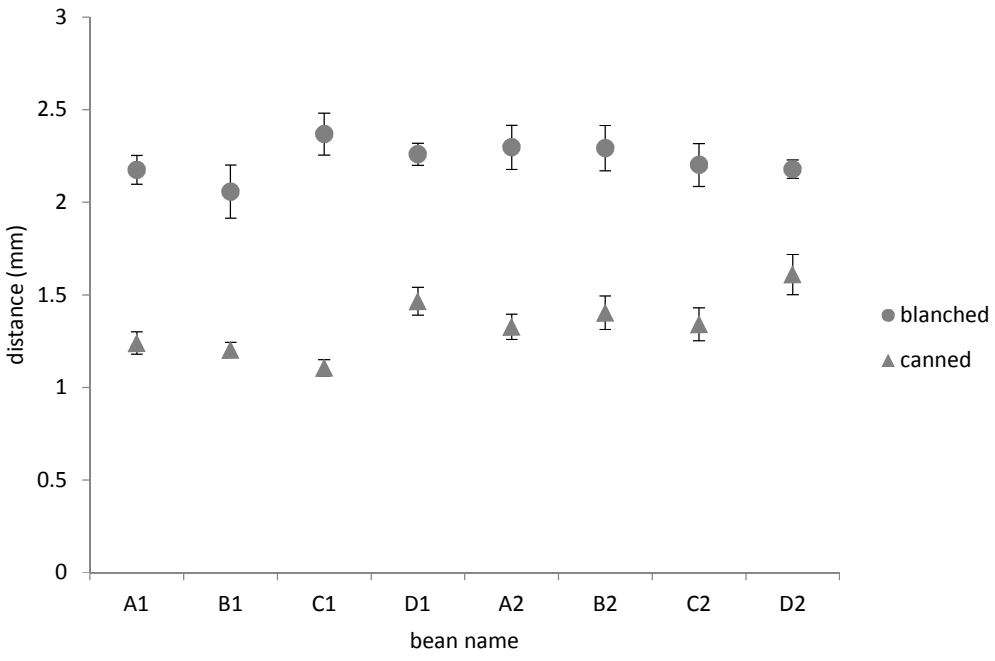

Figure 3.6 Deformation at first skin breakage for blanched and canned beans.

Values show mean of 10 replicates +/- standard error of mean at 95% confidence interval.

3.4 Mechanical properties of whole batch of canned beans (10g)

Investigation of the canned beans is crucial to the objectives of this project, as people consume the canned beans in a mouthful amount rather as single beans. The mechanical properties of a whole batch of canned beans are very important to understand the sensory properties. To assess the firmness of the whole batch of canned beans, Kramer shear cell and Ottawa cell were used to perform an extrusion measurement.

3.4.1 Kramer toughness

Figure 3.7 shows the curve obtained after the Kramer shear cell procedure. The Kramer shear cell is a multi-bladed device. The sample to be sheared is often of variable configuration or structure. The result is an average of the work required to cut through the sample of variable geometry. At point A, the blade contacted the surface of the bean sample. From A to B, the beans were deformed and compressed to pack more and more tightly into the diminishing space available under the blade, there is no rupture or breaking of the food. At approximately point B the beans were packed solid and liquid began to be compressed, beans filled up the space between blades. At point B or soon afterwards the pack was solid except for small amounts of entrapped air, the force increased steeply from B to C extruding any liquid out in the process. At point C, the beans began to rupture and extrude through the outlets. As soon as the beans samples went through the outlets, the force decreased. After the extrusion, parts of the samples were stuck between the blades and the outlets which made it was harder for the blades to go through the outlets so that there was a little increase at point D. The rest of the beans were extruded from the outlets after point D until the blades reversed direction and the force fell to zero at point E. Point C gave the force necessary to begin the process of extrusion, and the plateau CE shows the force needed to continue extrusion and shearing. From B to C represented the increasing force being applied to an almost incompressible mixture of solid and liquid. The positive area from A to E represented the work it needed to complete the extrusion and shearing process.

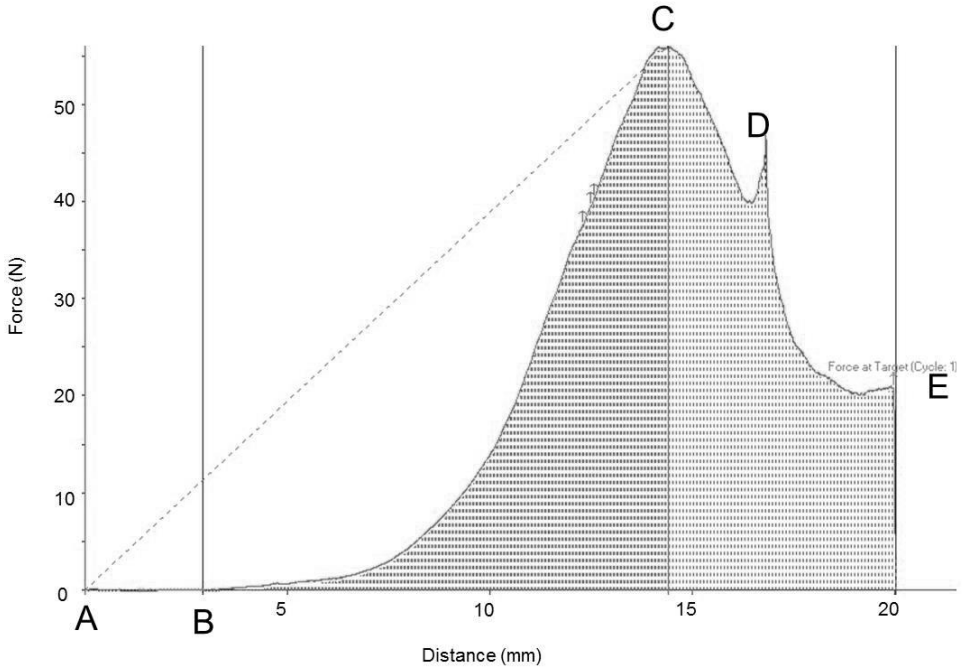

Figure 3.7 Diagram of the procedure of whole batch of beans (10g) performance using Kramer cell probe to assess the canned beans Kramer toughness.

3.4.2 Ottawa toughness

Figure 3.8 shows the curve obtained after the Ottawa procedure. The compression-extrusion test consists of applying force to beans until it flowed through the outlets. At point A, the compression plate contacted the beans surface, from A to B, force is seen to increase at a steady rate, the beans were packed down solid and the air between the particles was removed. As the plunger moves down further onto the sample the force begins to increase rapidly as the sample begins to deform and rupture. From B to C, after rupturing has occurred the subsequent increase in force is as a result of the force required to push and extrude the sample through the outlets. The area from A to C representative the work it needed to complete the compression and extrusion process, and it was obtained and used as an indication of firmness of the beans.

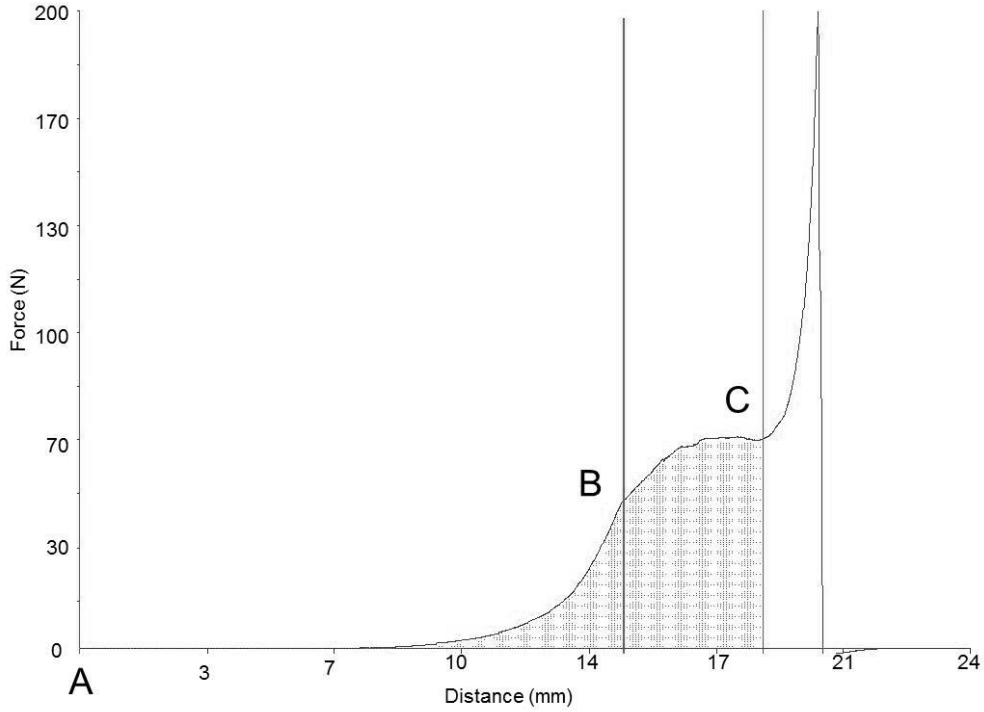

Figure 3.8. Diagram of the procedure of whole batch of beans (10g) performance using Ottawa cell probe to assess the canned beans Ottawa toughness.

In previous studies, the firmness was measured using an Instron Universal testing Instrument on different kinds of food products, such as pea, lentil, chickpea, navy bean, cowpea, green bean, red beans, and other vegetables and fruit such as carrot and cherry (Zhao and Chang, 2008, Proctor and Watts, 1987, Wang and Chang, 1988, Sefadedeh et al., 1978, Lu and Chang, 1996, Stolle-Smits et al., 2000, Abu-Ghannam, 1998, Lin and Schyvens, 1995, Anzaldua-Morales and Brennan, 1982). For navy beans, firmness has been measured using different equipment and methods. Voisey and Larmond measured the textural characteristics of baked beans using five objective techniques including Kramer shear-compression cell and wire extrusion/ Ottawa compression, back extrusion cell, the F. M. C Pea Tenderometer and plate extrusion cell (Voisey and Larmond, 1971). Navy beans were also reported to be measured with an Instron Universal testing Instrument (Wang et al., 1988, Lu and Chang, 1996); Kramer shear press was used to measure navy cooked beans (Walters et al., 1997, Lu and Chang, 1996, Balasubramanian et al., 2000, Anzaldua-Morales and Brennan, 1982) as well as other food products including green bean and lima bean (Stolle-Smits et al., 2000, Luh et al., 1975). Furthermore, Ottawa system

was reported to be used to test baked beans and various foods including cooked soybeans, canned green pears and cooked fish (Voisey, 1971).

3.4.3 Effect of cultivar and region on mechanical properties of whole batch of canned beans.

Table 3.9 shows the Kramer toughness and Ottawa toughness of canned beans when measured as a whole batch (10g). Numerically C2 was the toughest bean and A1 was the softest bean both for Kramer toughness and Ottawa toughness. C2 was 82% - 84% tougher than A1. In order to investigate the effect of the cultivar and region on the firmness of whole batch of canned beans, results were analysed stratified by growth regions and cultivars. Kramer toughness data agree to the assumption of a normal distribution, so that a two – factor ANOVA was carried out (Table 3.10). However, Ottawa toughness data did not fit a normal distribution, not even after data transformation. So that the non-parametric comparisons test - Kruskal Wallis test (Chapter 2, section 2.8.3) was carried out to compare the differences between beans (Table 3.11). For Kramer toughness, Table 3.10 shows that cultivar and region had significant effects on the Kramer toughness with interactions. Interaction was due to the beans from region B performed differently compared to the other three regions. In beans grown in region B beans, cultivar 2 was softer than cultivar 1, however in the other regions the results were opposite. Region affects beans toughness more than cultivar. C2 was significant tougher, A1 was significant softer. Beans from region C were tougher than region A and D, beans from cultivar 2 were tougher than cultivar 1 except region B. For Ottawa toughness, each type of bean was considered as an independent sample. Table 3.11 shows there was significant different between eight types of beans for the Ottawa toughness ($p = 0.005^{**}$), however, this test did not give more information for the differences between each individual type.

Table 3.9 Kramer toughness and Ottawa toughness when measure as a whole batch (10 g) of canned beans.

	Kramer toughness	Ottawa toughness
	N*mm	
A1	329.917[a]	274.907
B1	537.892[cd]	452.332
C1	477.175[bc]	449.851
D1	416.304[ab]	338.764
A2	435.440[abc]	367.377
B2	439.903[bc]	399.085
C2	615.241[d]	499.845
D2	456.306[bc]	367.658

Values show mean of 10 replicates. Letters a, b means within column with the same superscript letters are not significantly different at $p<0.05$ levels.

Table 3.10 Two factor ANOVA P-value for Kramer toughness of whole batch (10g) of eight types of canning beans.

	Kramer toughness
Cultivar	0.0087
Region	<0.001
Cultivar : Region	<0.001

At 95% confidence interval.

Table 3.11 Kruskal Wallis Test of Ottawa toughness of whole batch (10g) of canned beans.

Test Statistics[a,b]	Ottawa toughness (N*mm)
Chi-Square	20.347
Sig.	**.005****

a. Kruskal Wallis Test

b. Grouping Variable: bean.name

** Means significantly different at p<0.01 levels.

Figure 3.9 shows the relationship between Kramer toughness and Ottawa toughness, high linear correlation was found between them (r=0.95). The high correlation was because both methods were operating under similar mechanical conditions (extrusion measurement). Similar conclusion was obtained by Voisey and Larmond (1971), they measured the textural characteristics of baked beans using five objective techniques. And it was concluded that because of the high correlation and agreement between the different objectives, the hardness and cohesiveness of baked beans can be measured objectively using any of the methods (including Kramer shear-compression cell and wire extrusion/ Ottawa compression, back extrusion cell, the F. M. C Pea Tenderometer and plate extrusion cell) based on practical and economic considerations (Voisey and Larmond, 1971).

For academic study, Kramer shear cell is better to be used because the data obtained from Kramer had very clear peak and it was easy to collect area data, however for

the Ottawa, the probe touched the bottom of the plate in the end of the process so that the figure reached to a very high force in the end, but this part of data is not representative as the toughness of the beans, hence this area needed to be cut manually. Therefore, it is more convenient to use Kramer cell instead of Ottawa.

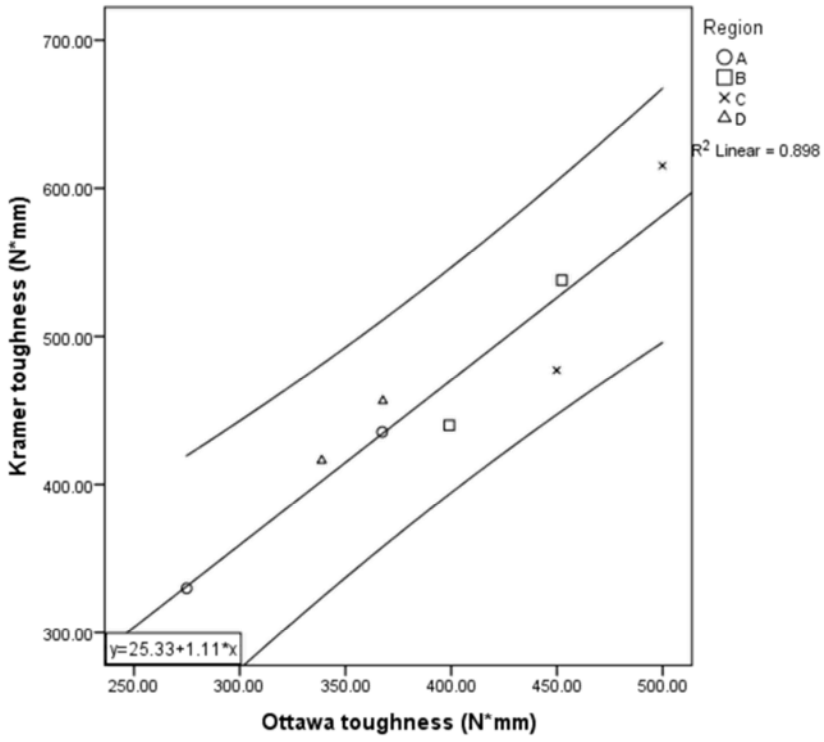

Figure 3.9. Relationship between average Kramer toughness and Ottawa toughness of whole batch of canned beans (10g) for eight types of beans.

Values show mean of 10 replicates. Arcs represent the 95% confidential intervals.

3.5 Rheological properties of the canned sauce

The sauce of canned beans is another very important factor when considering the quality of the canned baked beans. Because when consumers open a can of baked beans, the first image they have in sight is the sauce. Industrial customer feedback report showed that a thin sauce induced complains, which illustrated that sauce properties are a very important factor for the overall acceptance of the quality of canned baked beans. The ingredients of the sauce include tomato paste (7%, w/w%), sugar (5%, w/w%), granulated modified starch (1.6%, w/w%), salt (0.9%, w/w%), spirit vinegar (0.8%, w/w%), herbs and spices (0.51%, w/w%) and water (84.2%, w/w%).

Canned beans sauce was shown to be a non-Newtonian, shear thinning (pseudoplastic) liquid (Figure 3.10). Figure 3.11 shows that the viscosity decreased steeply with the increase of the shear rate from 0 to 20 (s^{-1}), and the it decreased more gently from 20 to 60 (s^{-1}), after 60 (s^{-1}) the viscosity stabilised with no big change. Because stimulus associated with the oral evaluation of viscosity is a shear stress developed in the mouth at an approximately constant shear rate of 50 (s^{-1}) (Shama and Sherman, 1973), the viscosity at shear rate 50 (s^{-1}) was collected to analyse the sauce mechanical properties. Figure 3.11 shows the firmest bean C2 had lowest sauce viscosity, with a highest absolute slope value from 0 to 40 (s^{-1}) shear rate. The softest bean A1 had highest sauce viscosity, with a lowest absolute slope value from 0 to 40 (s^{-1}) shear rate.

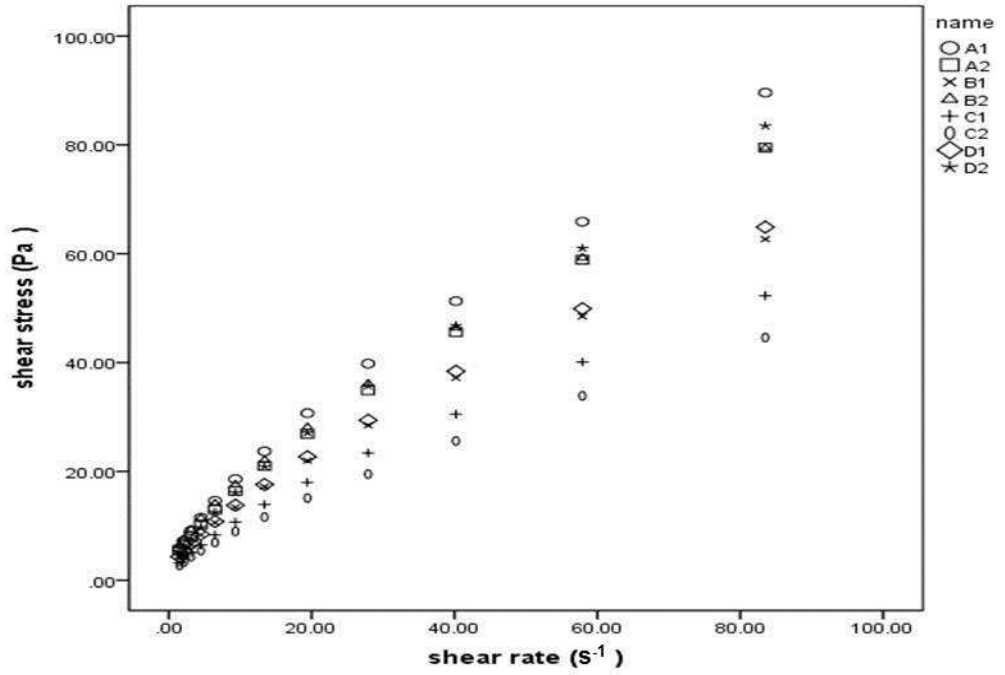

Figure 3.10 Rate of shear strain as a function of stress for different beans.

Values show mean of 3 replicates. s^{-1}

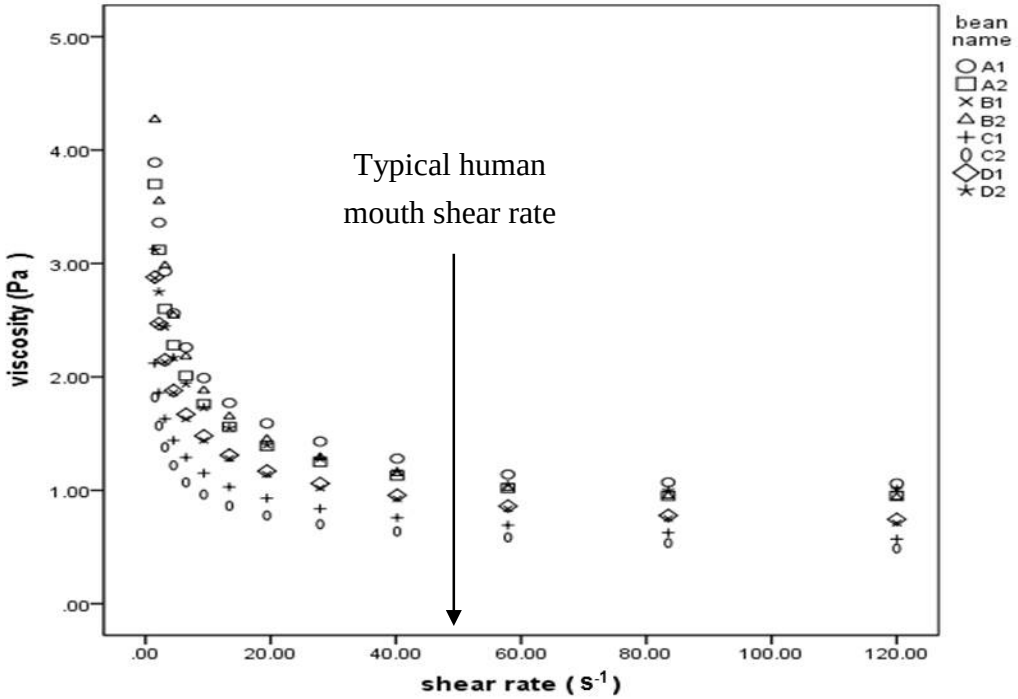

Figure 3.11 Dynamic viscosity of different beans at temperature of 25°C.

Values show mean of 3 replicates.

3.5.1 Effect of cultivar and region on the mechanical properties of the sauce of canned beans

Table 3.12 shows the viscosity of the sauce of canned beans. Numerically A1 had thinnest (less viscous) sauce and C2 had the thickest (more viscous) sauce. The viscosity of A1 was 94.9% thinner than C2. In order to investigate the effect of the cultivar and region on viscosity of sauce, two – factor ANOVA was carried out (Table 3.13). It shows that cultivar and region had significant effect on the viscosity of sauce with interactions. Interaction happened because in region A and C, beans from cultivar 1 had a thicker sauce than cultivar 2, however in region B and D, beans from cultivar 1 had a thicker sauce than cultivar 2. The opposite behaviour may cause the statistical interaction.

Table 3.12 Viscosity of the sauce of canned beans.

	Viscosity
A1	1.137^{d}
B1	0.836^{abc}
C1	0.692^{ab}
D1	0.861^{bc}
A2	1.017^{bc}
B2	1.024^{bc}
C2	0.585^{a}
D2	1.054^{cd}

Values show mean of 10 replicates. Letters a, b means within column with the same superscript letters are not significantly different at $p<0.05$ levels.

Table 3.13 Two factor ANOVA P-value for viscosity of the sauce of canned beans.

	Viscosity
Cultivar	0.0091
Region	<0.001
Cultivar : Region	<0.001

At 95% confidence interval

3.6 Effect of cooking medium on the texture quality of canned baked beans

Figure 3.12 shows appearance of the canned baked beans canned in water, brine (contained 0.7% salt and 4% sugar) and tomato sauce (ingredients see chapter 2.1.1). The colour of beans was lighter and the starchiness was less when canned in brine than in water. Both canning in water and brine caused large amount of splitting and cracking of the beans, however beans canned in tomato sauce were still holding their shapes.

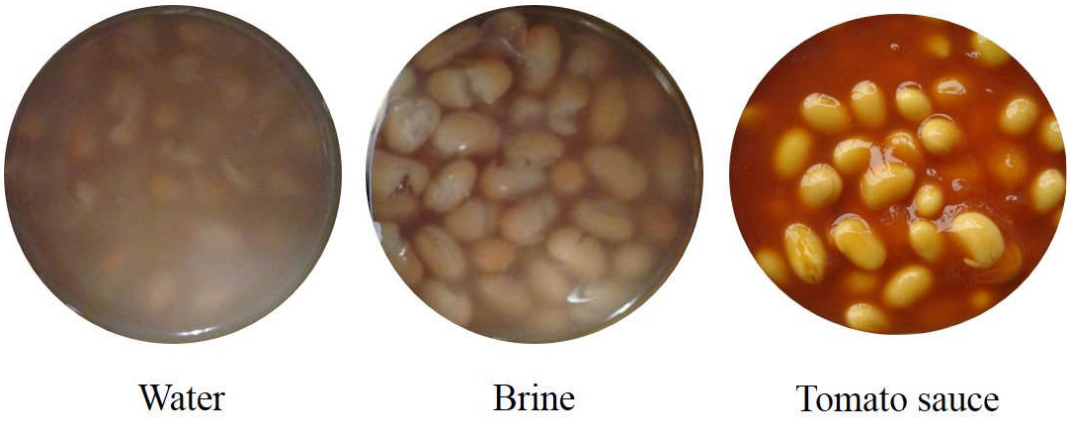

Figure 3.12 Beans canned in water, brine (contained 0.7% salt and 4% sugar), and tomato sauce (contained sugar, salt, starch, tomato paste).

The firmness of the canned beans processed in different media was measured. Figure 3.13 shows the Kramer toughness of a whole batch of beans (10g) using different canning media. Beans canned in tomato sauce were significant tougher than beans canned in water or brine (40% - 50%). Beans canned in brine were numerical tougher than canning in water but differences were not statistical significant. Thus, canning medium significantly affect the firmness of the beans, canning in tomato sauce produced firmer beans than water and brine. However, different conclusion were found by Anzaldua-Morales and Brennan (1982), they claimed that beans canned in tomato sauce were approximately 3 times softer than in brine using soft water (5% sugar and 1% salt) for compression, approximately 4 times softer for puncture, approximately 3 times softer for extrusion and 2.5 times softer for shearing (Anzaldua-Morales and Brennan, 1982). The differences can be caused by different canning methods from two projects, it may also cause by

different ingredients in the tomato sauce. Besides, the samples used in different project (cultivar, age etc.) may also had significant effect.

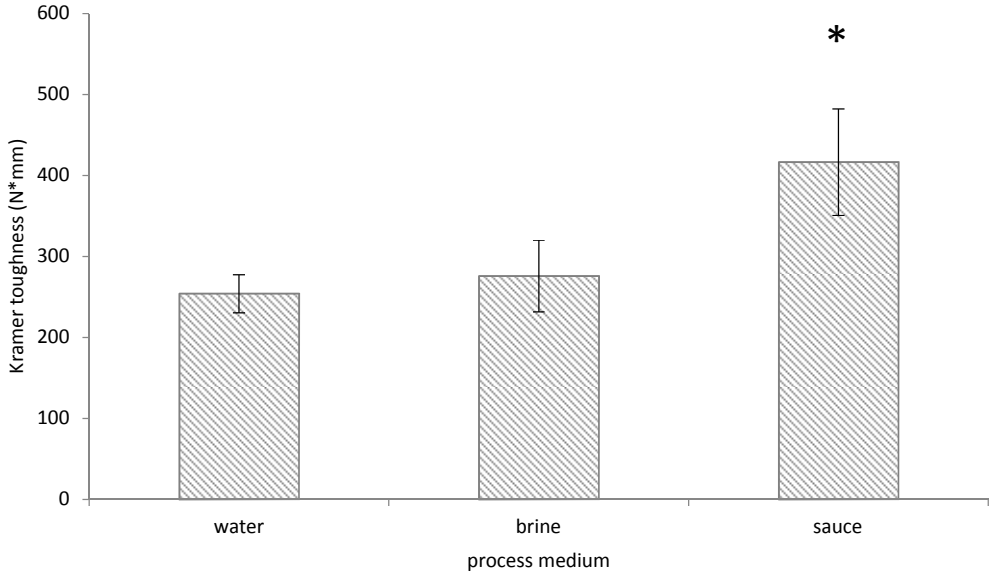

Figure 3.13 Kramer toughness of canned beans when measure as a whole batch (10 g) after different processing.

Values show mean of 3 replicates +/- standard error of means at 95% confidence interval. *Means significantly different at p<0.05 levels.

Figure 3.14 shows the viscosity of sauce from beans C2 canned in water and brine. It shows that there is no significant differences between them. Although from their appearance, sauce from canning in water seems more starchy than canning in brine (Figure 3.12). This indicated that the ingredients which made the medium seemed more starchy would not thicken the medium increasing its viscosity. The canned sauce is much more viscous (74 times thicker) than water and brine medium, however, because it included many other ingredients which would increase the viscosity (e.g. modified starch and tomato sauce), it is not comparable to water and brine medium. However, the tomato sauce components must have an effect on the beans, allowing them to preserve their shape.

There are some reports that discuss the cooking medium effect on the colour, splitting and firmness of the beans. Wang et al. in 1988 indicated that the addition of EDTA and calcium chloride would procedure lighter colour of navy beans as well as pinto beans, also the addition of calcium chloride to the brine increased the drained weight, increased the firmness of canned beans by 2 to 3 times, and reduced the

splitting and clumping (Wang et al., 1988, Wang and Chang, 1988). Although calcium chloride was not studied in this project, that the results show that cooking medium is a very important factor which would affect the canning quality.

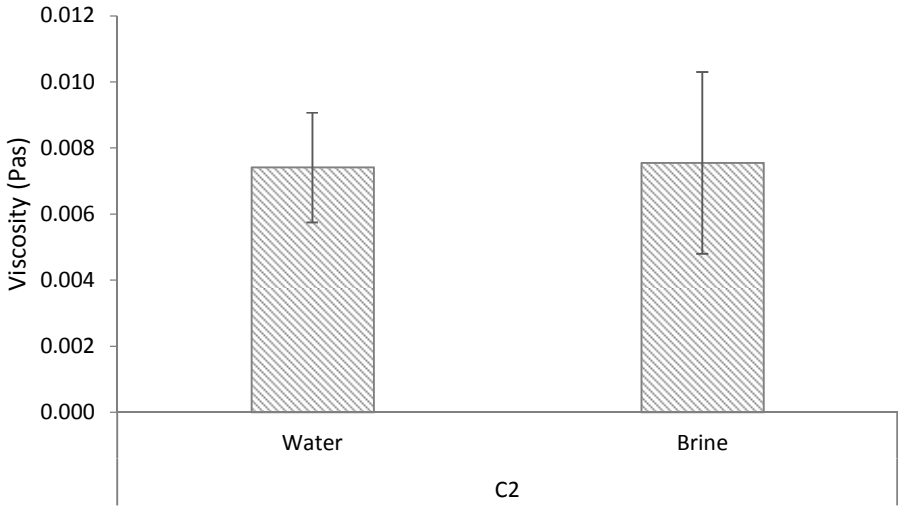

Figure 3.14 The viscosity of sauce of beans C2, after canning in water and brine.

Values show mean of 3 replicates +/- standard error of means at 95% confident interval.

3.7 Conclusion and discussion

Firmness of the beans is one of the most important properties of canned baked beans. The firmness of both single and a whole batch of beans were measured. For academic purposes, it is important to understand the mechanical properties of single bean's tissue such as the skin or cotyledon, because these measures can be associated with tissue ultrastructure and chemical composition, which will be the topic of the following chapters. However, at a practical level, analysis of single beans is time consuming, requiring a high number of replicates because of the high variability between beans of the same batch. For industry purposes, the mechanical properties of whole batch of beans is more important as customers consume mouthful portion of beans instead of single beans, and it can be better associated with data from sensory panels. Drained weight was suggested as a simple and useful measure of canning quality, as it discriminated between cultivars and regions. Any

of the methods developed in this chapter can be used to measure the firmness of the beans, and their correlation with each other and with chemical properties will be further explored in chapter 7. Which method is chosen depends on the purpose and circumstances. According to the results, the firmness of whole batches of beans gave a more accurate representation of the cultivar/region effects. This may due to the larger variance of the single bean data. As shown in Figure 3.15, although single bean data and whole batch data have similar precision, single bean data has larger variance (larger circle shown in figure) because of the biological variation. Therefore, data from whole batches of beans is better to distinguish the cultivar/region effect. The Kramer shear cell is preferred compared to Ottawa due to the analysis convenience (curve obtained is easier to collect data), however Kramer shear cell is very hard to clean due to the multiple blades.

For skin hardness, the data may have less precision than cotyledon data because the probe may puncture different position of the beans skin. So a fixed equipment is needed to ensure each test is puncturing the same position of the beans in order to increase the precision.

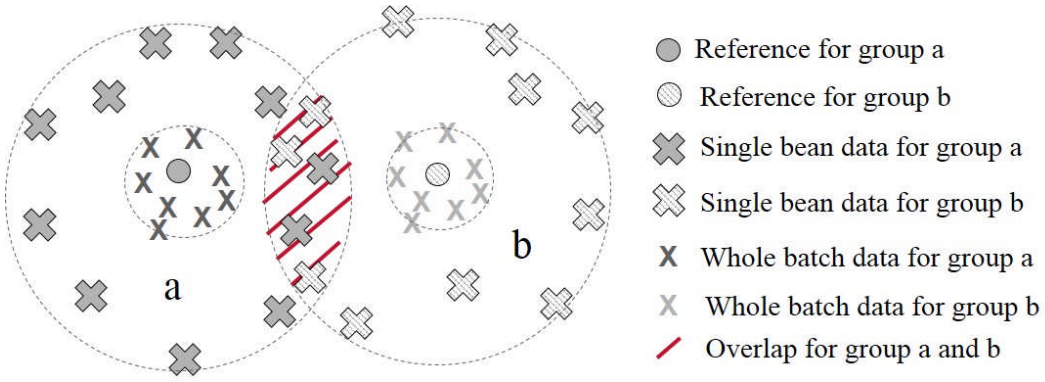

Figure 3.15 Demonstration of the distribution of data

Cultivar and region were found to have significant effect on the quality of canned beans, including the physical and mechanical properties. Cultivar was found to have significant effect on the hardness of blanched beans, while region had a significant effect on the firmness of canned beans. Interactions between cultivar and region were found for canned beans, indicating that some bean cultivars maybe most affected by region than others. Generally C2 was considered as the firmest bean, and A1 was considered as the softest bean. Their differences at the ultrastructural and chemical composition level will be explored in further chapters.

There have been few reports reporting the effect of cultivar and region on the texture of canned beans. Most of them found significant differences but one did not. Voisey and Larmond (1971) found there were significant differences in the firmness of canned beans from 4 different cultivars grown in Ontario (similar to region C) using Instron universal testing machine (with Kramer shear cell, back extrusion cell, a wire extrusion cell, a plate extrusion cell and a pea tenderometer). A high correlation and agreement between the different objective techniques was found, cultivar 779-629 was considerably softer than Seaway, Sanilac and Seafarer. Anzaldua-Morales and Brennan (1982) found differences in firmness of canned beans from 6 cultivars using Instron universal testing machine (with puncture, compression, Kramer shear and extrusion tests), beans from A had hardest beans and E had softest beans (cultivar and region were not specified). Walters et al. (1997) also found differences in firmness of canned beans from 3 cultivars (Seafarer, N81099, and Cumulus) grown in 2 different locations in Michigan using Kramer shear cell, and the study claimed that, cultivar, region as well as growth year all significantly affected the firmness of canned beans with interactions, region and growth year were of higher influence. None of these papers provided structural or biochemical explanations for their observations. However, Lu and Chang (1996) correlated chemical composition (soluble pectin yield) and canning quality of beans from 11 cultivars from two locations in North Dakota (similar to D), using Instron universal testing machine with Kramer shear measurement. They found no significant effect of location on firmness, and although differences between cultivars was implied from their discussion, the paper did not present the firmness data for the different cultivars. The paper did show that more pectin could be extracted from softer beans than from firmer beans. In the case of this project, the cultivars were not specified because of commercial confidentiality issues. But the regions where the beans grown were roughly known. One possible explanation for the differences observed can be attributed to climate. Region A and B (centre of North America) are known to have extreme climate characteristics (short humid summer and long cold winter). Region C (East-Central Canada) is classified as humid continental (hot summer with high humidity level). Region D (upper Midwestern of the United State of America) has hot semi-humid summers and cold dry winters. It is believed that high temperature and high humidity would induce hardening of the legumes during storage, and seed hardening has been associated with reduction in the solubility of uronic acid and

arabinose rich pectins. (Stanley et al., 1990, Shiga et al., 2004, Liu and Mcwatters, 1994). The climatic information supports the observation that beans from region C had the firmest texture and beans from A had the softest texture. Region C has typically the hottest and most humid climate, which may be associated with the hardening of the beans. But region A has longest winter and coldest weather, which is less associated with inducing beans hardening. However, future study needs to be done to prove the hypothesis that climate affects bean physiology, and also to investigate other determining factors such as the composition of the soil, the amount of rainfall and longitude and latitude. The information also supports the role of pectic polysaccharides in bean mechanical properties, which will be discussed further in paragraphs below.

Thermal processing dramatically softened the texture of the beans. It has been suggested that the reduction in firmness is highly associated with the breakdown of the middle lamella during cooking (Sefadedeh et al., 1978). Therefore, it is believed that the microstructure of the beans changes in some way to soften the texture. In the next chapter, the effect of processing on the microstructure of the beans will be investigated. In addition to the properties of the beans, the properties of the sauce are also very important for consumer's acceptance of the product. In this chapter, differences in viscosity were found between found amongst the different beans samples, with significant effects of both cultivar and region. Interestingly, the beans do not lose their coherence during thermal processing, indicating that most of the bean components stay in the bean. This may be due to the presence of hydrocolloids in the sauce (starch and tomato fibres) that prevent the leaching of bean components. When the beans are canned in brine or water, tissue disruption and leaching of 'starchy' material is apparent. This starch material is likely to be bean starch from bean cells that have been ruptured due to high osmotic pressure. However, the starchy material did not appear to affect viscosity, maybe due to low gelatinization properties. This suggests that the presence of the tomato sauce is essential for optimal textural quality. The solubility of the bean polymers will be investigated in chapters 5 and 6.

Chapter 4
Microstructure of navy beans

4.1 Introduction

The results from chapter 3 indicated that the mechanical properties of the skin and cotyledon are affected by cultivar, region and processing conditions. These differences may be due to the organization and/or structure of the tissue, which in turn are determined by the composition and organisation of the cell wall. Therefore, this chapter will investigate the tissue organisation and microstructure of the cell wall, and for the first time visualise the effect of cultivar, region and processing conditions on the cell wall polysaccharides of the beans from the localization microscopies.

In this chapter, the aims were: 1) to characterize the general tissue organisation and cell wall microstructure of the skin and cotyledon of a typical bean; 2) to investigate the effect of growth region, cultivar on microstructure of the cell wall; 3) to investigate the effect of thermal processing on microstructure of the cell wall; 4) to investigate the effect of different cooking medium on the tissue organisation of beans.

Figure 4.1 shows the diagram of simulation of a bean canning in tomato sauce, showing the structure of navy beans which conclude skin and two cotyledons. Both of microstructure of the skin and cotyledon was studied. The microstructure of the cell wall was investigated by localizing cell wall components (cellulose, pectic polysaccharides, extensin and mannans) by cytochemical and immunocytochemical microscopy of skin and cotyledon sections of raw, blanched or canned beans. Cell wall ultrastructure was investigated using fluorescent specific probes observed by epifluorescence microscopy. Calcofluor white dye was used to localize cellulose, while anti-pectin, anti-mannan and anti-extensin antibodies were used to localize specific cell wall components. General tissue structure was characterised by toluidine blue staining of membranes on the sections, observed by contrast light microscopy.

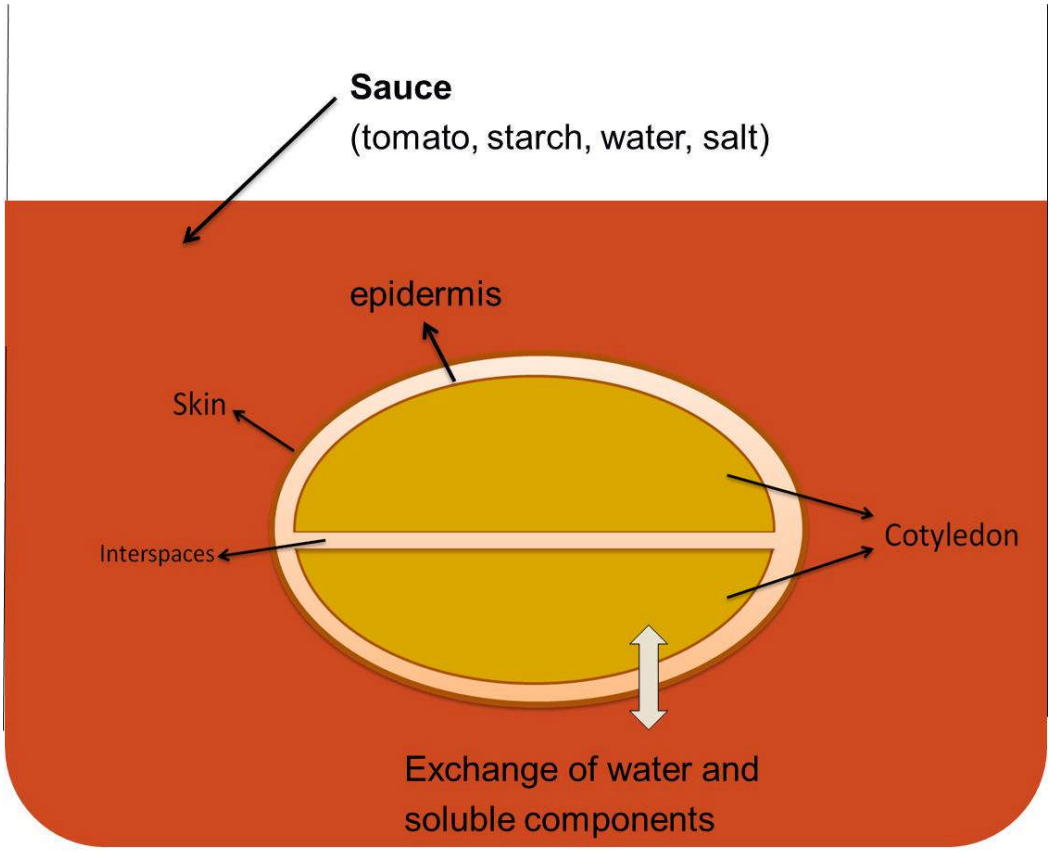

Figure 4.1. Schematic diagrams of simulation of a bean canning in tomato sauce, showing the structure of the navy bean.

4.2 Characterisation of a typical navy bean skin

4.2.1 General microstructure of the skin

Skin tissue (seed coat) structure differs among species and varieties. Transverse section of a sample of bean skins showed variable thickness, ranging between approximately 150-200µm. Figure 4.2 shows a diagram illustrating the anatomy of bean skin. There are four layers in a typical bean skin, which are cuticle, macrosclereids, ostosclereids and parenchyma. The outermost layer is the waxy cuticle, it represents the very first barrier to the external environment. The waxy cuticle decreases water loss and control gas exchange (Clark et al., 1995). Proximal to the cuticle layer is a single stratum of thick-walled, elongated palisade columnar macrosclereids cells. These cells are tightly bound to each other without any inter cellular spaces. The next layer is composed of a single layer of thick-walled ostosclereids, cells are separated by wide intercellular spaces. The fourth layer is the parenchyma, formed by several layers of thin-walled parenchyma cells which are organized into cell layers of small – big – small cells.

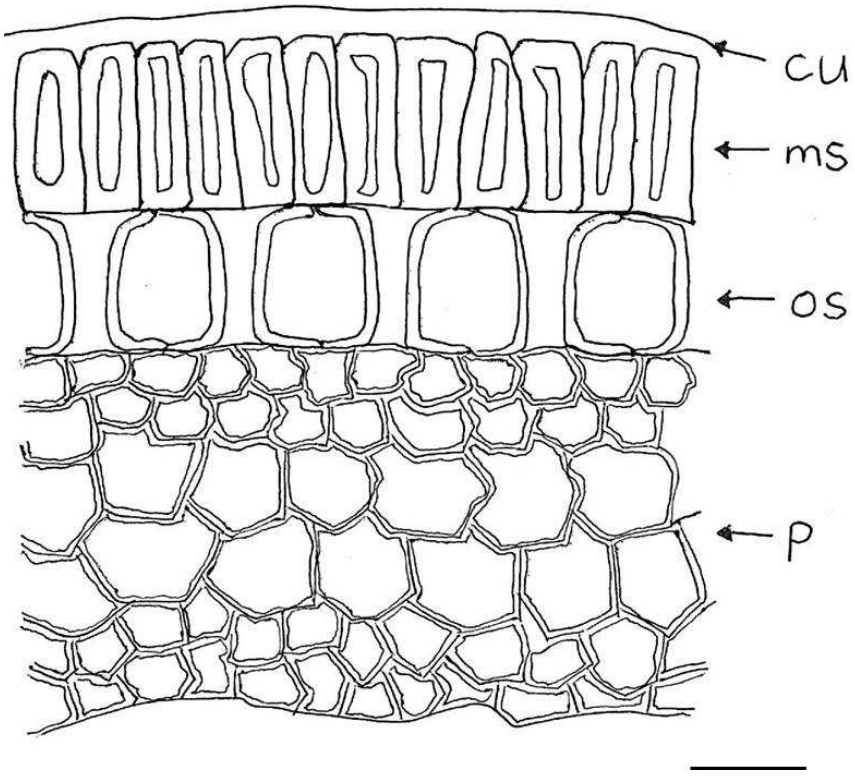

Figure 4.2. Anatomy of a typical bean skin (seed coat).

cu=cuticle; ms=macrosclereids; os=ostosclereids; p=parenchyma. Scale bar =25µm.

To visualise the general microstructure of the skin, toluidine blue staining for light microscopy. Calcofluor White staining for UV microscopy was applied to localize cellulose in the cell walls (a typical bean skin is shown in Figure 4.3). Four layers of cells in the skin were observed. The cuticle layer was very thin. The thickness of macrosclereids layer (27 – 35 μm) was slightly thicker than osteosclereids layer (15 – 23 μm). The outer macrosclereid layer was abundant in cellulose, in contrast to the osteosclereid layer (Figure 4.3 B). The more abundant cellulose in macrosclereid layer may contribute to the strenge of the skin, provided the majory protection against outside world. Proximal to the osteosclereid layer is a layer of parenchyma which contain approximately 10 – 14 cells in thickness, inner and outer parenchyma cells were usually smaller than the central cells (Figure 4.3 B). In the inner parenchyma (closer to cotyledon layer), more cellulose were detected than the other layers (Figure 4.3 B), it may indicated that the inner parenchyma was stronger than the other layer of parenchyma. Because of the cells in parenchyma layer were lossely attached to each other, they were easily lost during the preparation for microscopy (Figure 4.3 A). No starch was observed in the skin section. Hence the turgor pressure will not change too much after water imbibition, which made the skin structure more stable compare to cotyledon which contained large amount of starch.

Some reports classified skin layers based the seed development. For example, McCartney and Knox (2002) considered the pea testa parenchyma layer as two layers which are inner parenchyma layer and outter parenchyma layer, the inner of which is crushed at maturity so that it is also called crushed parenchyma layer. Albornoz classified the layer for Fabaceae seeds from colubrina var. cebil tree as a small layer of inner osteosclereid, two or three strata of small macroesclereids and an inner cuticle separating the seed coat from endosperm (Varela and Albornoz, 2013). Therefore, the seed coat (skin) structure is described differently amongst different varieties, especially the parenchyma layer. This is the first time that navy bean skin mictostructure has been investigated. This structure may be important for determining the mechanical properties of the tissue.

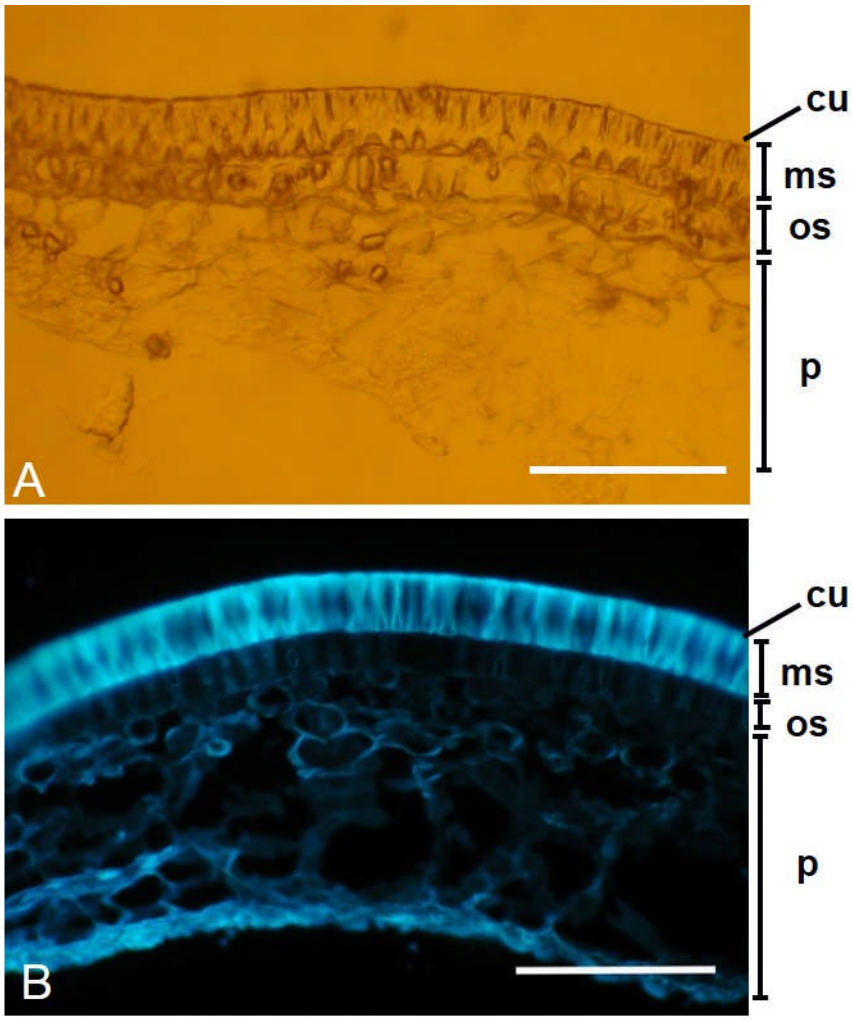

Figure 4.3. Respresentative micrographs of sections of navy beans skin. Section stained with toluidine blue and observed under light microscopy (A), and section stained with calcofluor white and observed under UV light (B).

Micrographs show the structure of navy bean skin and localisation of cellulose. cu=cuticle; ms = macrosclereids; os = osteosclereids; p = parenchyma. Scale bar for all micrographs = 100µm

4.2.2 Localization of cell wall pectic polysaccharides in skin

Pectic polysaccharides are abundant in cell walls (middle lamella and primary wall), and contribute to cell adhesion and tissue cohesion, they also have a role in controlling cell wall porosity and mechanical strength (Caffall and Mohnen, 2009, Wang et al., 1988, Lu and Chang, 1996). Therefore, pectin in the navy beans were investigated to study its role in influencing the texture of the beans. The localization of different cell wall pectin were investigated in section of raw beans that soaked in

formaldehyde to fix proteins. Four monoclonal antibodies were used to label four different types of pectic epitopes. JIM5 was used to label un-methyl-esterified HG, JIM7 was used to label methyl-esterified HG, LM5 was used to label galactan and LM6 was used to label arabinan (Chapter 2, section 2.3). Primary antibody labelling was coupled to a second antibody conjugated to fluorescent FITC. Immunofluorescence labelling of a typical skin tissue with the four antibodies is shown in Figure 4.4. Bean D1 represents a typical bean structure. Un-esterified HG (labelled with JIM5) was found to be abundantly present throughout all the navy bean skin cell walls especially in the osteosclereids layer (Figure 4.4 B). Navy raw bean skin was also abundant with highly methyl-esterified HG (labelled with JIM7), galactan (labelled with LM5) and arabinan (labelled with LM6) (Figure 4.4 C, D and E), although not as much as un-esterified HG. All of these three types of pectin epitopes were present mainly in macrosclereids layer followed by inner parenchyma, and less in the other layers (Figure 4.4 C, D and E). The macrosclereids layer and inner parenchyma layer appeared to have more cell wall polysaccharides (pectin and cellulose) which may play protective roles for the skin. Less galactan and arabinan were detected than HG in parenchyma layer (Figure 4.4).

Navy bean skin has not been studied by visualising its structure before. Other species skin has been reported like pea. McCartney and Knox (2002) found that methyl-esterified HG was abundant in pea testa but with low levels of un-esterified HG, and both were not dependent on the stage of the development. Galactan and arabinan occurred in restricted and distinct locations: galactan was detected in the macrosclereids layer and arabinan occurred in the inner parenchyma cells. And both of galactan and arabinan were dependent on the developmental stage of the seed. This discovery was quite different from navy beans, un-esterified HG was detected abundantly present throughout the entire navy bean skin cell walls especially osteosclereids layer (Figure 4.4B). Thus, different from pea testa, un-esterified HG played a dominant role amongst pectins in navy bean skin. If calcium was present, the un-esterified HG would be cross-linked through calcium-pectin bridges in the middle lamella promoting cell adhesion, also lead to a firmer texture.

Galactan and arabinan epitopes was found to have different locations and were separate from HG (Orfila and Knox, 2000). The localization of galactan and arabinan in navy bean skin was also different from pea testa (McCartney and Knox, 2002). Although the seed development stage factor was not studied for navy beans,

the galactan and arabinan were not found to be localized in restrict and distinct regions of the cell wall in skin, as is the case in pea testa. They are presented through all the cell walls especially the macrosclereids layer. Although the functions of pectic galactan and arabinan components are not clear, it is suggested that they are all significantly related to development stage or the position or maintenance of pit fields (Orfila and Knox, 2000). Therefore in the future study, it would be of interest to investigate the presence of different pectins at different development stages of navy bean.

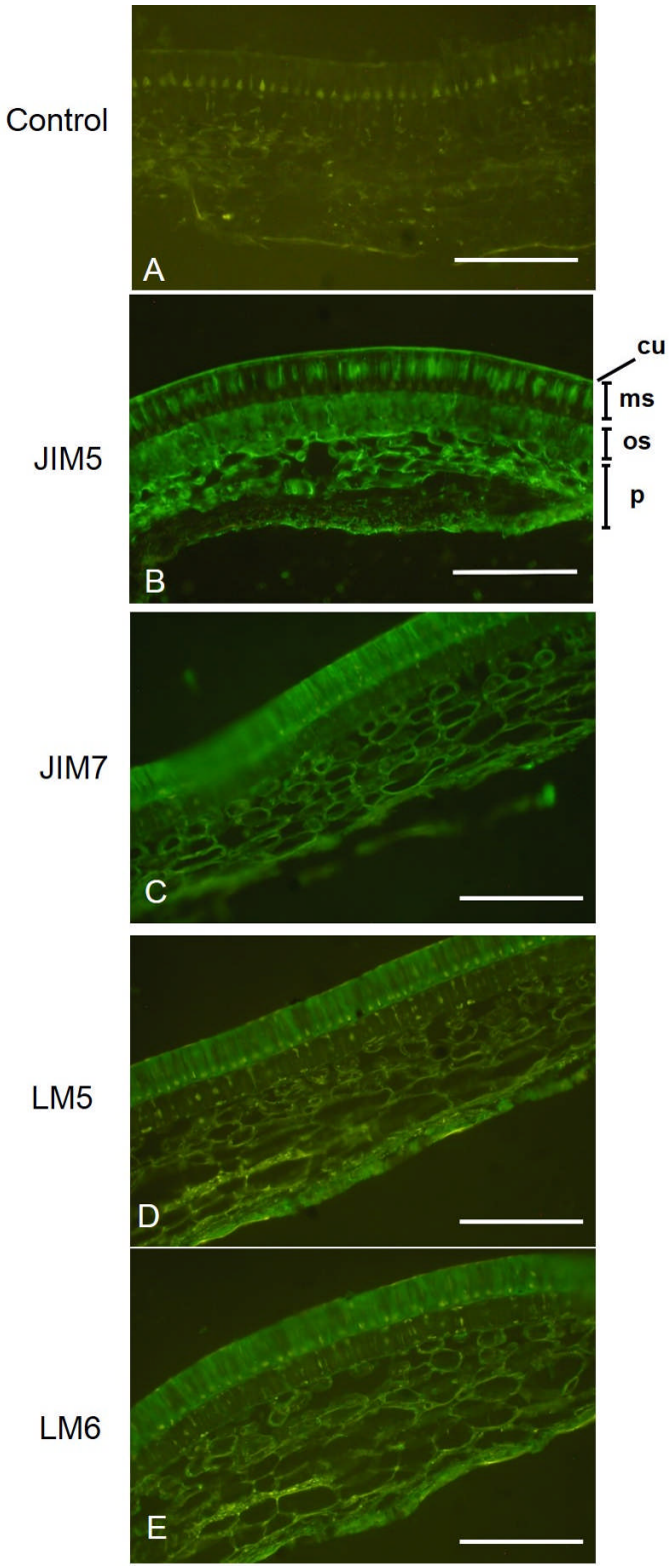

Figure 4.4. Respresentative micrographs of skin sections of raw bean, control treatment without antibody (A), labelled with antibody JIM5 (B), JIM7 (C), LM5 (D) and LM6 (E).

Showing localization of un-methyl-esterified HG, methyl-esterified HG, galactan and arabinan respectively. cu=cuticle; ms = macrosclereids; os = osteosclereids; p = parenchyma. Scale bar for all micrographs = 100µm

4.2.3 Effect of thermal processing on the localization of pectic polysaccharides of the skin

After canning, the skin hardness decreased dramatically from 15N to 1N. Figure 4.5 shows the skin of bean C2 after canning. Raw bean skin showed an intact structure with four distinct layers, however canned bean skin lost the osteosclereids and parenchyma layer. As discussed above, macrosclereids layer was composed of tightly attached cells which abundant with cellulose and pectin, so that macrosclereids layer was believed to be relative firmer than the other layers. Although osteosclereids and parenchyma layer were rich in pectin, they composed of loosely attached cells. Therefore, after heat treatment, loosely attached cells seperated apart and lost in the sauce, only left the firmest organisation – the macrosclereids layer which is still recognisable. Raw bean skin was abundant with un-esterified HG with highly fluorescence, however canned bean skin shows hardly any HG labelling, most of the pectin was believed to lost into the sauce during the canning. In short, canning significantly affected the skin texture by destorying osteosclereids and parenchyma layer and solubilising the pectins in macrosclereids layer. Because canned skin was too soft and very hard to collect and prepare, so the data only shows the localisation of JIM5 epitopes but no other antibodies.

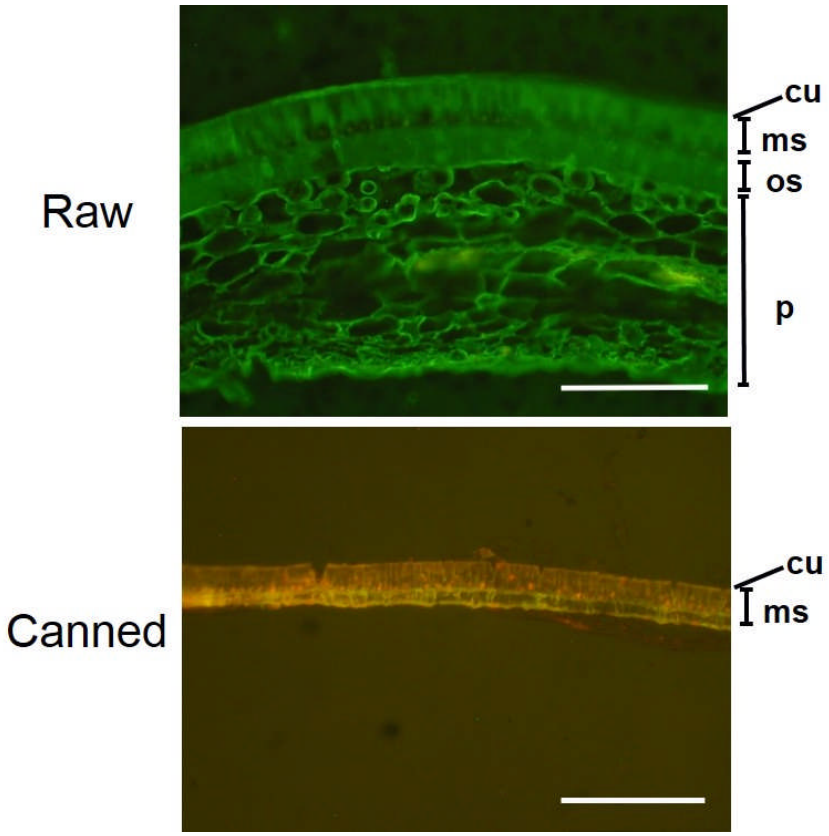

Figure 4.5. Respresentative micrographs of skin sections of raw and canned navy bean, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5.

Showing the location of un-methyl-esterified HG in the cell wall. cu=cuticle; ms = macrosclereids; os = osteosclereids; p = parenchyma. Scale bar for all micrographs = 100µm

4.2.4 Effect of cultivar and region on the localization of pectic polysaccharides in the skin

In order to investigate the effect of cultivar and region on the cellulose localisation in the skin, eight types of raw bean skin were prepared for microscopy and stained with Calcofluor white (Figure 4.6). All beans presented abundant cellulose staining. It had brighter cellulose staining in two parts: the macrosclereids layer and the inner parenchyma cells (Figure 4.6). To understand the role of cellulose on texture, the softest and toughest beans were compared. A1 was considered as the softest bean and C2 was the firmest bean. The palisade columnar structure can be clearly seen in C2 macrosclereids layer, however A1 appeared to have a more diffuse structure. Cellulose staining in C2 was brighter than A1 in the macrosclereids layer. The cuticle layer can be clearly seen in C2 but not in A1. Thus, tougher beans were found to have a more structural clear macrosclereids layer which presented more cellulose, the differences are quite subtle. However, no obvious pattern was found for the effect of region or cultivar on cellulose staining for eight types of beans.

In order to investigate the effect of cultivar and region on the pectic polysaccharides of the skin, raw bean skin were prepared for microscopy and labelled with antibodies. Figure 4.7 shows that un-methyl-esterified HG presented throughout the entire cell wall in the skin, no distinct differences or obvious pattern can be found amongst the different beans. Although soft beans A1 shows more diffuse structure in the macrosclereids layer, the binding for A1 and C2 did not have big difference. Hence, cultivars and regions did not found to have significant effect on the localization of un-methyl-esterified HG in skin tissue. No significant effect was found for other pectic epitopes (JIM7, LM5 or LM6) either (data not shown).

Therefore, no distinguish differences on microstructure of skin were found amongst eight types of beans, although subtle diffuse structure was found in soft beans. Neither staining for cellulose nor labelling for pectin were found to have any differences amongst different beans either. It is possible that this method can not quantify small differences but only significant ones, such as processing difference (4.1.3) or development difference, such as the pea testa (McCartney and Knox, 2002). Hence, no effect of cultivar or region on skin texture were visualized by immunofluorescence and staining mehod.

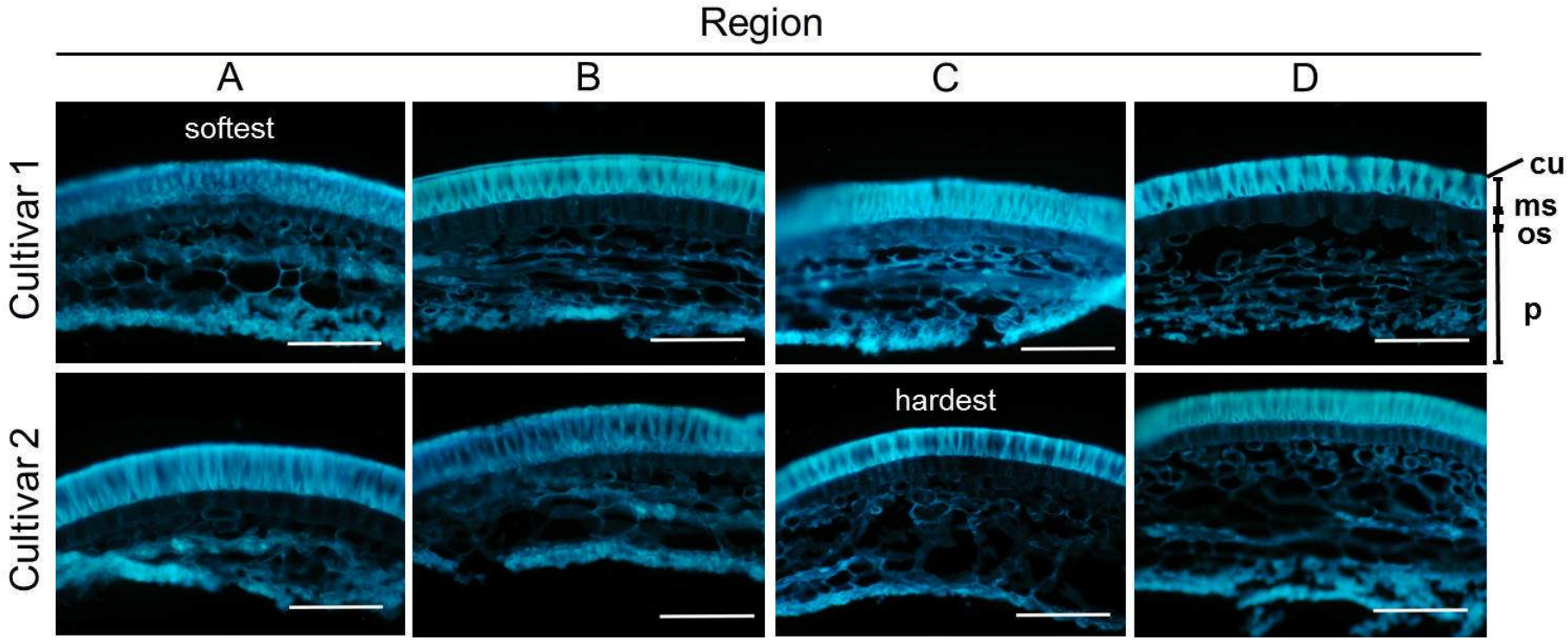

Figure 4.6. Respresentative UV micrographs of skin sections of raw bean soaked in formalyhyde, stained with calcufluor white, from two cultivars and four regions.

Showing the location of cellulose in the skin. cu=cuticle; ms = macrosclereids; os = osteosclereids; p = parenchyma. Scale bar for all micrographs = 100µm

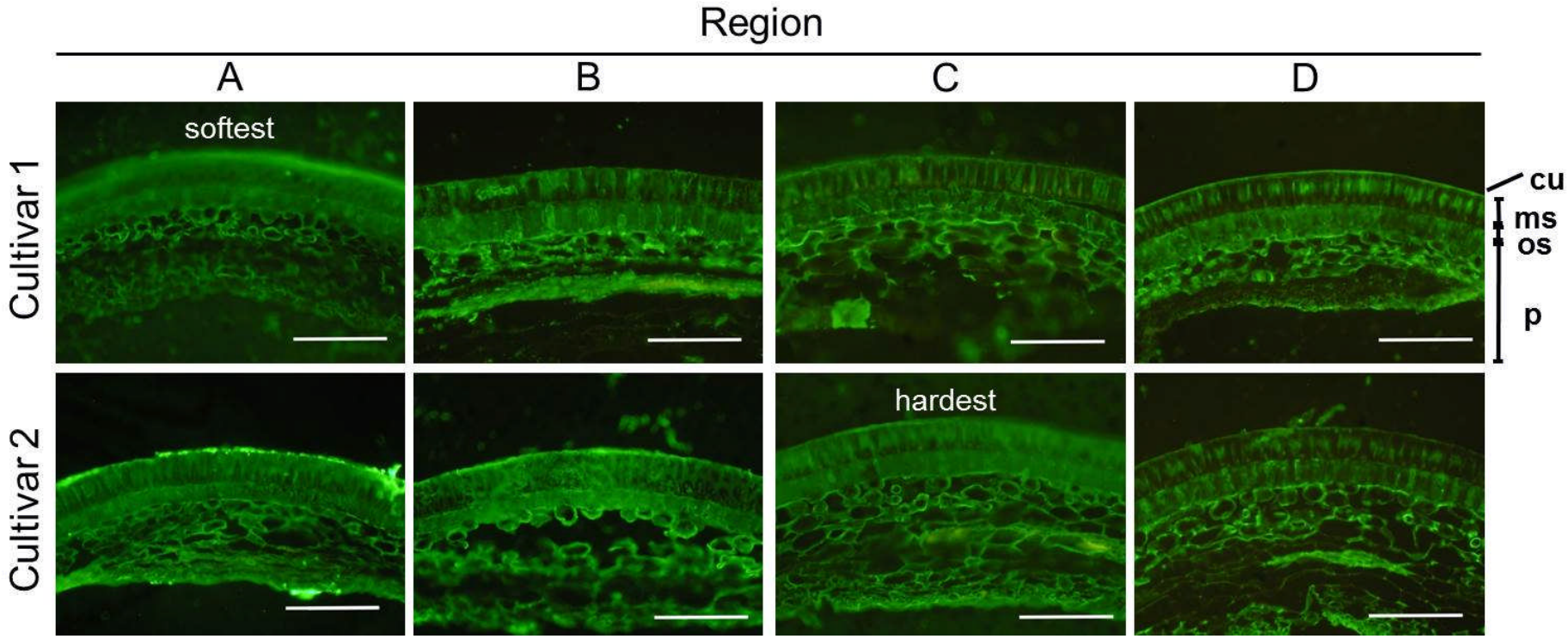

Figure 4.7. Respresentative micrographs of skin sections of raw bean soaked in formalyhyde from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5.

Showing the location of un-methyl-esterified HG. Scale bar for all micrographs = 100µm cu=cuticle; ms = macrosclereids; os = osteosclereids; p = parenchyma. Scale bar for all micrographs = 100

4.2.5 Localization of the other cell wall components in the skin

Cellulose microfibrils are embedded in and cross linked to a network of hemicelluloses (including xylans, mannans, xyloglucans, glucurononmannan, galactomannan etc.). Proteins are crossed linked in the cell walls among cellulose and hemicellulose. In this section, another two probes were used to localised hemicellulose and protein in the cell walls. Monoclonal antibody LM1and LM21 has been derived to have affinity for extensin and heteromannan respectively (Smallwood et al., 1995, Marcus et al., 2010). Extensins are a family of hydroxyproline-rich glycoproteins (HRGPs) which are abundant in dicotyledonous primary cell walls, and are more complicated than in monocotyledonous plants (Smallwood et al., 1995). Heteromannan is a form of mannan which consists of two or more different monomers of mannose, galactose or glucose. LM21 was found to bind to mannan polysaccharides in the primary cell wall, and secondary wall of some legumes which contain abundant galactomannan as storage polysaccharides (Marcus et al., 2010, Albersheim et al., 2011). In this project, localization of extensins and heteromannan was briefly examined in the skin.

LM1 and LM21 epitopes were not detected in the skin (Figure 4.8). Both pictures are auto-florescence but no binding was detected. Hence extensin and mannan cannot be visualised in raw navy beans skin. Marcus et al. (2010) indicated the detection of mannans can be effectively blocked by the presence of pectic homogalacturonan in the primary cell wall. Hence, the non-detection of the mannans was possible caused by the abundant existence of HG in the skin. One way to unmask mannan polymers is by enzymatic removal of pectic HG (Marcus et al., 2010).

Although skin is of great interest, it did not contribute to the overall texture as much as cotyledon. Skin has a much thinner structure compare to cotyledon, so it requires much less force to break. The customers evaluate beans texture / firmness mainly according to the cotyledon but not skin. Meanwhile, for practical purpose, skin samples were extremely hard to prepare and easy to lose. Therefore, for the above reasons, in this project, the microstructure of skin were only briefly investigated, and focus on study the cotyledon microstructure.

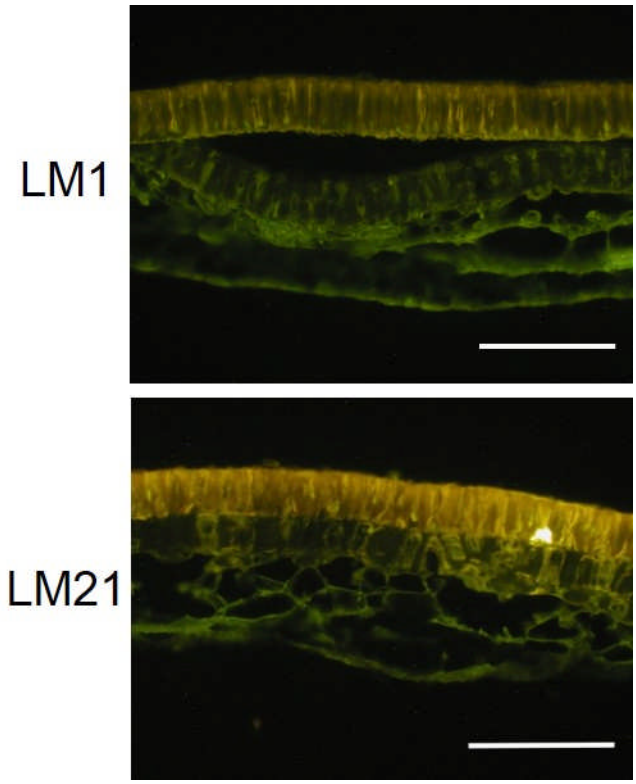

Figure 4.8. Respresentative micrographs of skin sections of raw bean soaked in formalyhyde, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM1 and LM21.

Showing the location of extensin and heteromannan respectivly. Scale bar for all micrographs = 100µm

4.3 Characterisation of a typical navy bean cotyledon

Firmness is one of the most important factors that influence consumers' acceptance of canned beans (Lu and Chang, 1996). The firmness of the canned beans was primarily determined by cotyledon toughness. Hence the properties of cotyledon are very important for understanding the texture of canned beans. In this section, the cotyledon microstructure was studied. There are some reports reporting the cotyledon microstructure of dry beans using scanning electron microscopy (Rockland and Jones, 1974, Hahn et al., 1977, Berg et al., 2012, Stolle-Smits et al., 1998). However, it is the first to investigate the ultrastructure of navy beans cotyledon by UV and immunofluorescence microscopy methods in this project.

4.3.1 General structure of the cotyledon

Navy bean is a dicotyledonous plant. Each cotyledon transverse is composed of two layers, which are epidermis and parenchyma. Figure 4.9 shows a representative parenchyma cell structure, showing the primary wall, middle lamella and intercellular space. Plasmodesmata can be seen in primary pit-fields. Primary walls are deposited generally as a thin, flexible and extensible layer which contains cellulose, pectin, hemicellulose, protein and phenolic compounds. The middle lamella is a pectin-rich layer that joins together the primary walls of adjacent cells. The spaces between cells adhesions are called intercellular spaces, usually presented as a triangle shape.

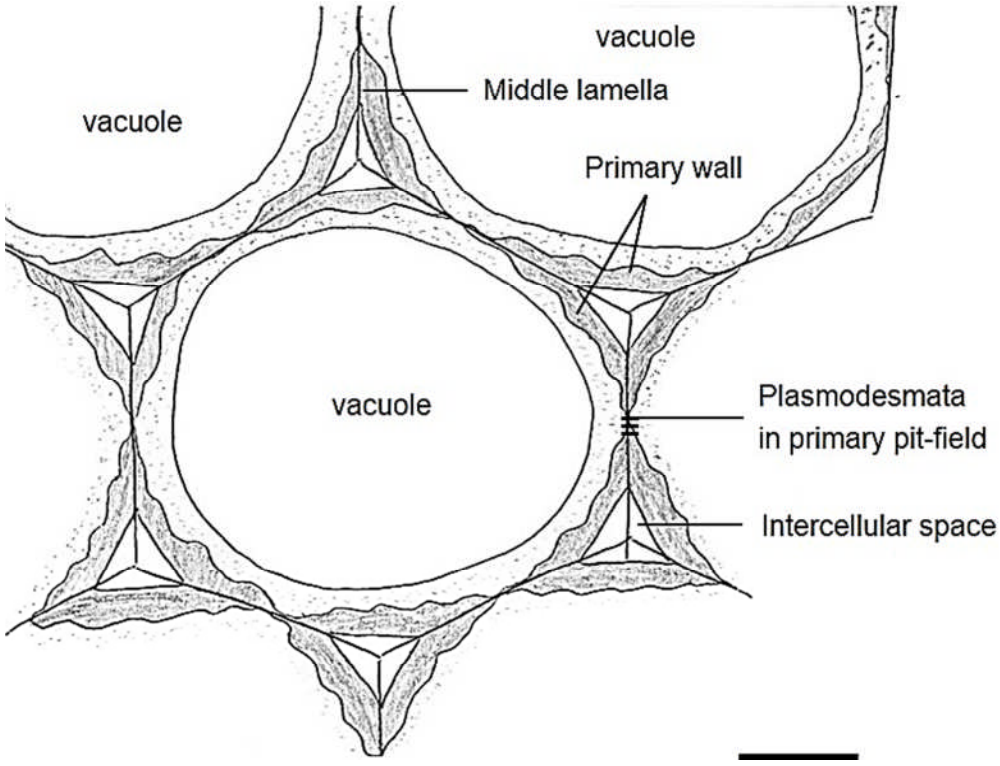

Figure 4.9 Diagrammatic representative of a typical plant cell, showing the primary cell wall structure of cells. Scale bar = 20µm

To visualise the general microstructure of the cotyledon and localise cellulose in the cell walls, toluidine blue staining for light microscopy and Calcofluor White staining of cellulose for UV microscopy were undertaken (a typical bean cotyledon is shown in Figure 4.10. Two layers were observed which were epidermis and parenchyma. The outer layer near the skin is the epidermis, it is composed of a single layer of small, tightly attached cells that are 11µm in size. Besides the skin, the epidermis also represents a barrier for the inside parenchyma cells. The epidermal cells

contained no starch granules. The other layer is parenchyma, it is composed of several layers of parenchyma cells that are 60µm – 110µm in size, hexagonal shape and full of starch granules. The parenchyma cell walls were the focus object in this section.

Look at individual parenchyma cell (Figure 4.10). The region between the two primary cell walls of adjacent cells is middle lamella. The non-adhesion small space among primary walls formed intercellular space. Pit field can be seen from the cellulose structure of beans (Figure 4.10 C and D). Cytoplasm (Figure 4.10 B and D) consist of cytosol, membrane-bounded entities (organelles such as plastid and mitochondria), system of membranes (endoplasmic reticulum and Golgi apparatus), and nonmembranous entities (such as ribosomes, actin filaments, and microtubules) (Raven et al., 2012). Starch, the primary storage polysaccharides in plants, are stored as starch granules within plants. The size of the starch granules range from 20µm to 50µm. The starch granules in the pictures are non-fully hydrated granules. Starch granules expand after taking in water, and cell wall constrains expansion of the protoplast and prevents rupture of the plasma membrane when the protoplast Localization of cell wall pectic polysaccharides in cotyledon enlarges following the uptake of water by the cell (Raven et al., 2012).

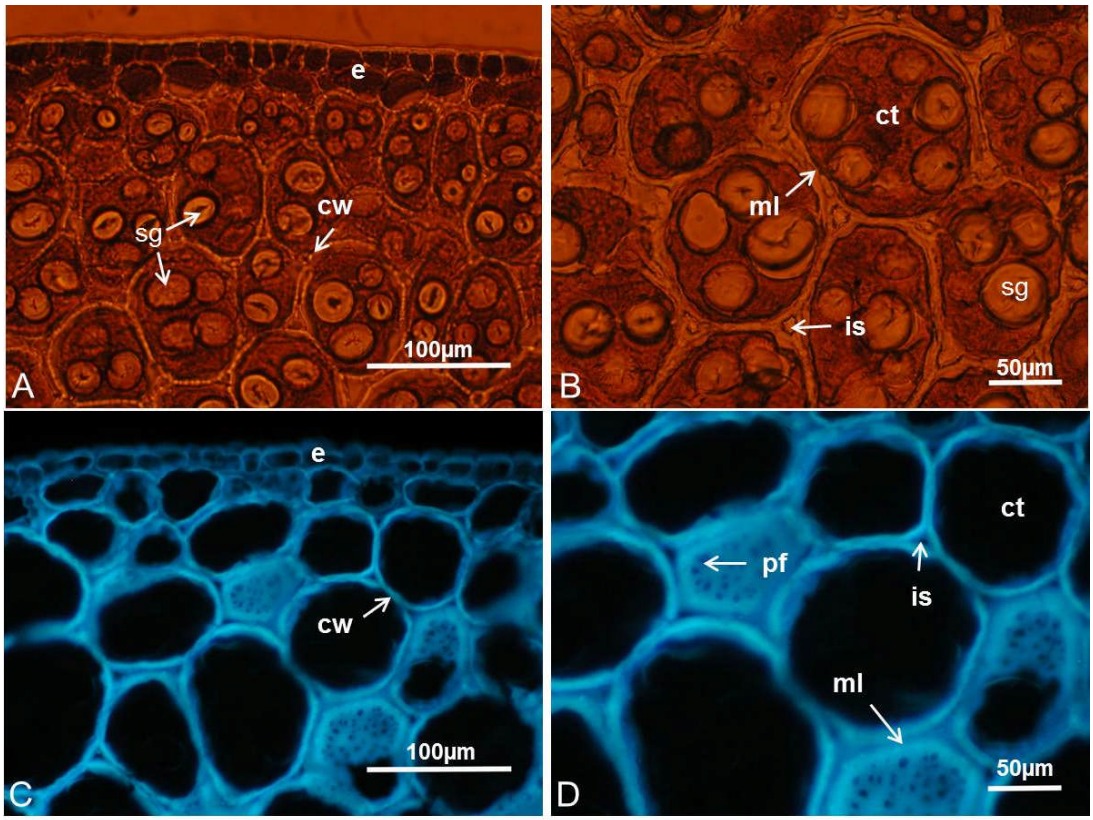

Figure 4.10 Respresentative micrographs of raw navy bean cotyledon soaked in formalyhyde, light micrographs of sections stained with Toluidine blue (A) (B), UV micrographs of sections stained with calcufluor white (C) (D) with different magnification.

Showing the cells details of the navy bean and cellulose of the navy bean. is = intercellular spaces, cw = cell wall; ct = cytoplasm; ml = middle lamella; e = epidermis; sg = starch granule; pf = pit field.

4.3.2 Localization of cell wall pectic polysaccharides in cotyledon

The localisation of different cell wall pectin in cotyledon sections were investigated in this section as for analysis of skin. Four monoclonal antibodies were used which were JIM5, JIM7, LM5 and LM6. They labelled epitopes un-methyl-esterified HG, methyl-esterified HG, galactan and arabinan respectively. Primary antibody labelling was coupled to a second antibody conjugated to fluorescent FITC. Figure 4.11 shows the labelling of un-methyl-esterified HG and methyl-esterified HG binding by JIM5 and JIM7. Intense binding of JIM5 can be seen throughout the primary cell walls, and it was much stronger in the primary wall and corners of intercellular spaces than the other regions of the cell wall. JIM7 binding was weaker

than JIM5 binding, but similar as JIM5 binding, stronger fluorescence was detected in the corners of the intercellular spaces and primary walls. Large amount of HG was detected in in navy cotyledon especially un-methylesterified HG. Un-methylesterified HG are usually crossed linked by calcium. It is know that HG-calcium complexes can be resulting in gel formation, and contribute to cell walls strength and cell intercellular adhesion (Caffall and Mohnen, 2009, Willats et al., 2001b). Therefore, the abundant HG in cell wall may contribute to cell adhesion and thereafter contribute to the beans firmness.

Other species have been studied using same methods and probes. For JIM5 binding, similar detection was found for carrot root by Knox et al. (1990), JIM5 epitope has been localized to the inner face of the cell wall adjacent to the plasma membrane. Intense labelling occurred at the surface of walls exposed at intercellular spaces. Bush et al. (2001) indicated that un-methyl-esterified HG epitopes are densely labelled in the middle lamella of the potato (Bush et al., 2001). For JIM7 binding, different results were found from Knox in carrot root, JIM7 was located evenly throughout the cell wall in all observed instances however navy beans was more strongly labelled in the middle lamella and corners of intercellular spaces, this may indicated that the cell adhesion was more strong in navy beans than carrot root, so that navy beans texture were general firmer than carrot. Therefore, it showed HG was abundant in plant primary wall and middle lamella and contribute to cell adhesion.

Figure 4.12 shows the labelling of galactan and arabinan binding by LM5 and LM6. Compared to JIM5 and JIM7, LM5 and LM6 binding was much weaker. Galactan epitope (LM5) appeared to be restricted to an inner region of the parenchyma and epidermal cell walls, located in a narrow region of the primary cell wall close to the plasma membrane. Labelling of intercellular spaces and middle lamella cannot be seen. In contrast, arabinan epitope (LM6) was more widely distributed throughout the primary cell walls, but also more abundant in the inner region of the primary wall close to the plasma membrane. Similar as LM5, there was less binding in middle lamella and intercellular spaces. Epidermal cell wall presented more arabinan than galactan.

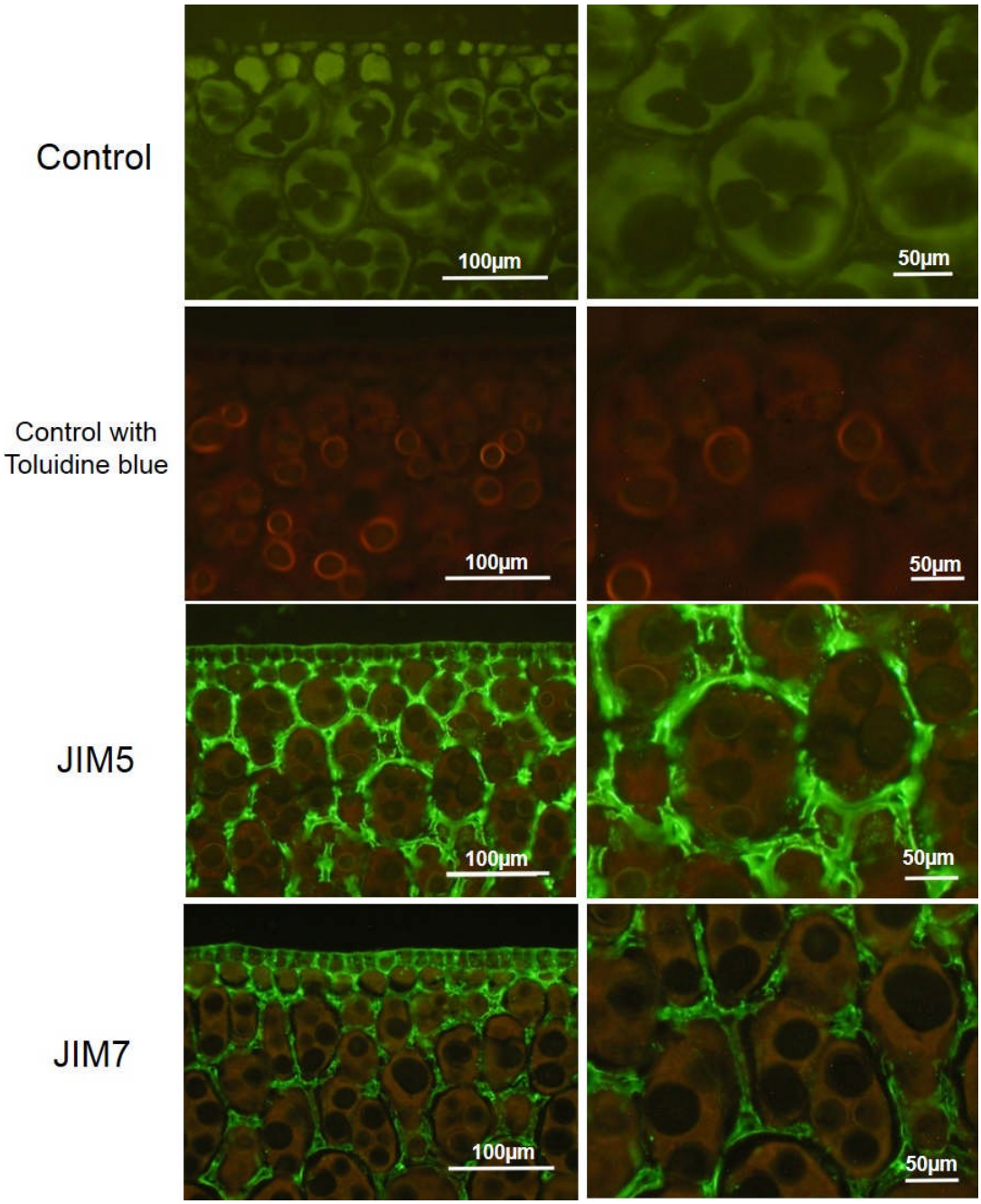

Figure 4.11 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5 and JIM7, with pre-treatment of Toluidine blue.

Showing the location of un-methyl-esterified HG and methyl-esterified HG respectivly.

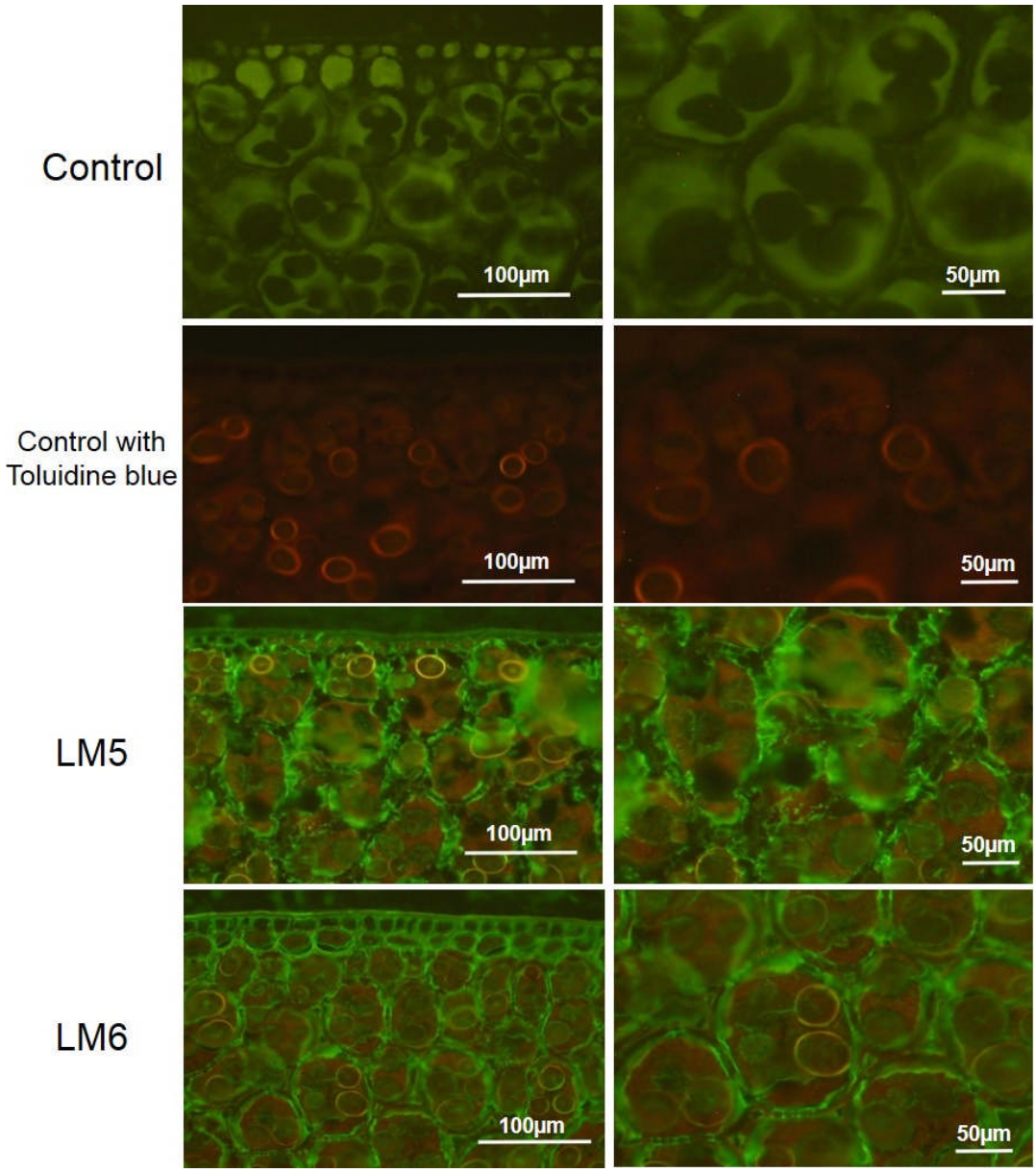

Figure 4.12 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM5 and LM6, with pre-treatment of Toluidine blue.

Showing the location of (1→4)-b-D-galactan and (1→5)-a-L-arabinan respectivly.

The distribution of galactan and arabinan epitopes in relation to cell wall architecture at intercellular spaces is different in different species, and varied for different development stages (McCartney et al., 2000). McCartney et al. (2000) claimed that, for lupin (*Lupinus angustifolius*) which are known to have massively thickened cell walls with galactan as a major component, galactan occurs throughout the cell wall thickening that surround the intercellular spaces, however, for peas, galactan is restricted to an inner region of the parenchyma and absent from the thickened cell walls surrounding intercellular spaces, so for pea it is unlikely to be a storage polysaccharides. Arabinan was only detected in a thin central region between adhered cell wall thickenings coincides with middle lamella in lupin. However arabinan was present in cotyledon cell walls of pea throughout development (McCartney et al., 2000). Therefore, pectic galactan and arabinan are suggested significantly associated with development stage and species (Orfila and Knox, 2000, McCartney and Knox, 2002). In this project, different development stages were not analysed (fully mature beans were analysed), so that the changes in the pectin distribution throughout the all the development stages were not known. But it can conclude that in raw navy beans, galactan was found to present in a thin inner region of cell wall and arabinan was present throughout the primary wall, but both of them were not intensely present in the intercellular spaces. It will be interesting to investigate the effect of development stage on the localization of pectin in the cell wall in the future study.

4.3.3 Effect of thermal processing on the microstructure of beans

Canning changed the beans texture significantly. The cotyledon toughness decreased dramatically by 83% - 89%. The thermal and cooking medium could be the factors that had effect on the texture and consequently influenced the microstructure. In this section, the thermal processing including blanching and canning; the cooking medium including water, brine and tomato sauce were studied in order to investigate the effect of processing on the microstructure of bean cotyledon.

4.3.3.1 Characterisation of a typical navy bean cotyledon after blanching and canning

Figure 4.13 shows the microstructure of cotyledon after blanching and canning. Figure 4.14 shows the diagram of intercellular space between three cells. Jarvis (1998) indicated that from when the intercellular space first appears to the cells eventually become round, the angle θ increases from zero to $\pi/2$. There are internal pressures which are exerted by turgor and cell adhesion force (Jarvis, 1998). When the turgor pressure is stronger than cell adhesion force, the cells separate, otherwise of the cells will burst (Jarvis, 1998).

Therefore, from the theory above. After blanching, the beans were not thoroughly hydrated and the cotyledon was still very firm. The cells adhered to each other tightly, cell adhesion force is stronger than turgor pressure. So it needed bigger force to break through the cells than break the cell wall. Hence the cell wall broke a lot when sectioning (Figure 4.13 C). After canning, the cells swelled, the starch granules expanded and filled in the cells (Figure 4.13 B). The cell shape changed from hexagon to round (Figure 4.13 C and D). The canning process loosened the middle lamella, the adhesion force is weaker than turgor pressure and lead to cell separation without breaking the cell walls.

The canning process caused cell separation, and consequently softening the beans texture. When customers consume the canned beans, the force needed to break the cell walls is bigger than the force to break the intercellular matrix, hence the force break the cell adhesion first instead of break the cell walls, which generate the sandy texture for the sensory analysis.

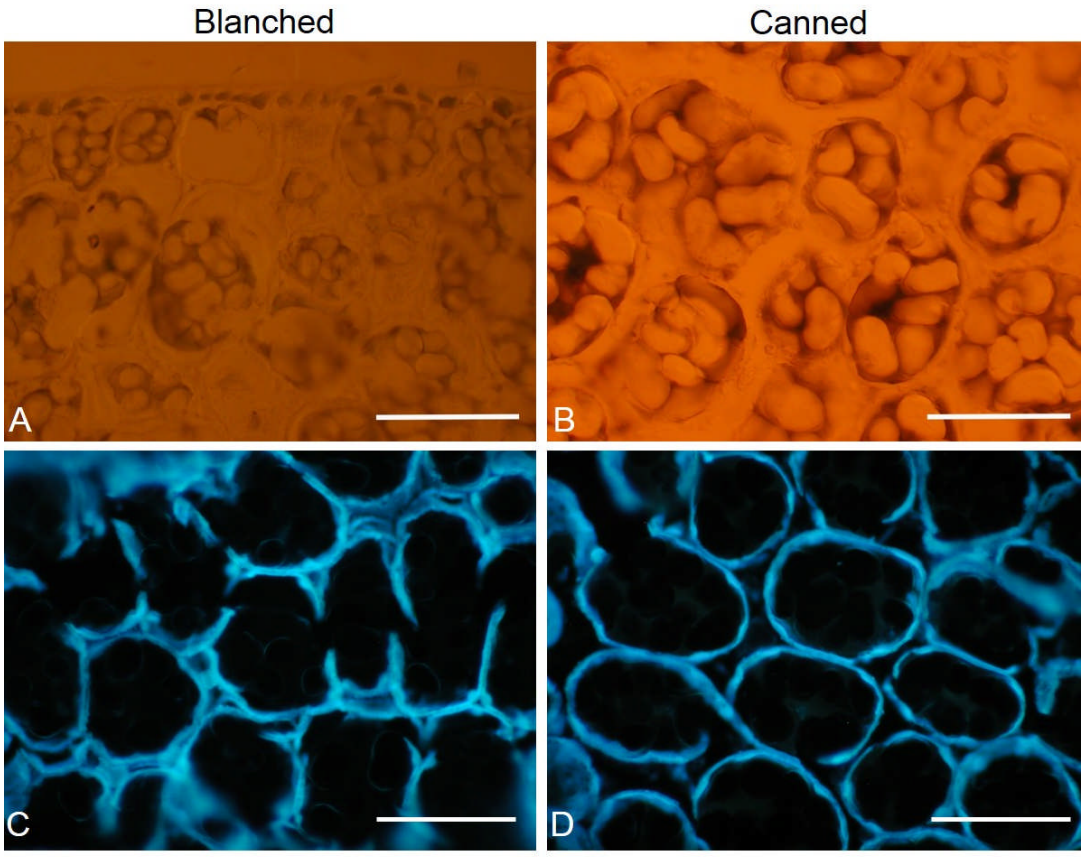

Figure 4.13 Respresentative micrographs staining of sections of navy beans cotyledon under light microscopy staining with Toluidine blue (A) and (B), UV microscopy staining with calcofluor white (C) and (D) after different processing, blanched beans (A) and (C) and canned beans (B) and (D).

Showing the details inside the beans and cellulose changes of the beans after canning. Scale bar for all micrographs = 100µm.

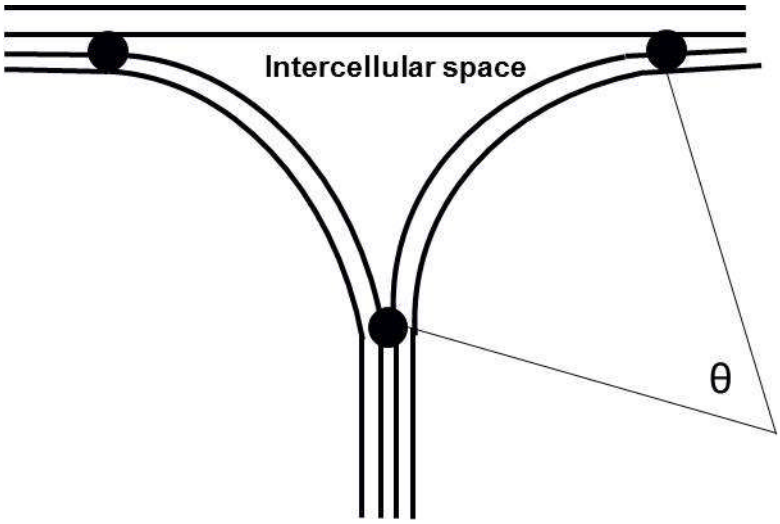

Figure 4.14 Tricellular junction with intercellular space between cells. (Adapted from (Jarvis, 1998))

4.3.3.2 Effect of thermal processing on the localization of pectic polysaccharides in the cotyledon

Canning process permitted separation of adjacent whole cells, presumably be weakening forces between materials within the middle lamella. To investigate the thermal processing effect on the pectic polysaccharides, labelling with antibodies of sections of canned beans was undertaken. Figure 4.15 shows the representative JIM5 labelling of the cotyledon under different processing. Raw beans cotyledon had strong JIM5 epitopes detection but blanched beans had less strong binding. This was first presumed to be relevant with enzyme activities; however no enzyme activity was found in the blanched beans (data not shown). So that the less labelling may because the hydration degree affected the antibody binding. Raw beans were soaked in formaldehyde overnight (12h) and were in a high degree of hydration, but blanched beans blanched in hot water (80°C) for only 20min so that they were in a low degree of hydration. So the antibodies did not have much substrate to react with antigens for blanched beans and result in a less binding of epitopes. It is also possible that part of the pectin lost during blanching and caused less labelling. To test if it was due to the loss by solubility or the hydration degree, beans can be blanched in longer time until it is fully hydrated. If the labelling increase, it means the less labelling of blanched beans caused by the hydration degree. If the labelling decrease, it means the less labelling of blanched beans caused by the loss by solubility.

After canning, the cell walls are highly hydrated, increasing access of the antibodies to the epitopes. Cells were round and separated. Different from the raw and blanched beans, the epitopes binding were detected evenly throughout the opened-up middle lamella instead of strongly binding in the corners of the intercellular spaces (Figure 4.15). The un-esterified-methyl-HG was abundant in the intercellular matrix. These pectin epitopes can be from two sources. Some of it was pectin from bean which was not lost into the sauce, the rest were believed came from the pectin from tomato sauce. Exchange of solute happened between beans and tomato sauce during canning process, so it is possible that beans soaked in some pectin from the tomato sauce and kept in the intercellular matrix. In the future study, in order to confirm where the pectin mainly came from, beans canned in water can be sectioned to investigate how the antibody epitopes detected.

Blanching is essential for good quality of canned beans, un-blanched and low temperature blanched beans would cause firmer beans (Davis et al., 1980, Anthon and Barrett, 2006), however, blanching did not seem to affect the localization of un-methylesterified HG in cell wall. Canning process significantly affected the localization of un-methylesterified HG in cell wall mainly by changing the cell adhesion. The adhesion strength is influenced by ripening and cooking (Jarvis, 1998), Orfila et al. (2001) studied the influence of ripening to cell adhesion, they indicated that during the tomato ripening, middle lamella HG was altered resulting in the cell wall swelling and softening, cell adhesion decreased. Arabinan was disrupted deposition and may contribute to the lack of pericarp softening. The influence of cooking to cell adhesion was also reported in cowpeas. Heating from 25 – 90 °C had no major effect on the microstructure. When the temperature was above 100 °C, fracture occurred in the middle lamella leaving most of the cell intact (Sefadedeh et al., 1978), and similar changes for lima beans were also found (Rockland and Jones, 1974). This may result from depolymerisation and solubilisation through heating. The major contribution to intercellular adhesion may come from the chelator-soluble pectin rather than water-soluble pectin (Walter and Taylor, 1991). Therefore, regardless of the ripening of beans, it is possible that heat treatment depolymerised the navy bean pectin in the middle lamella and intercellular spaces consequently allowing loosening of the middle lamella and leading to a softer texture. However, the depolymerize was not studied in this project, the depolymerisation of the polysaccharides need to detect further to be proved.

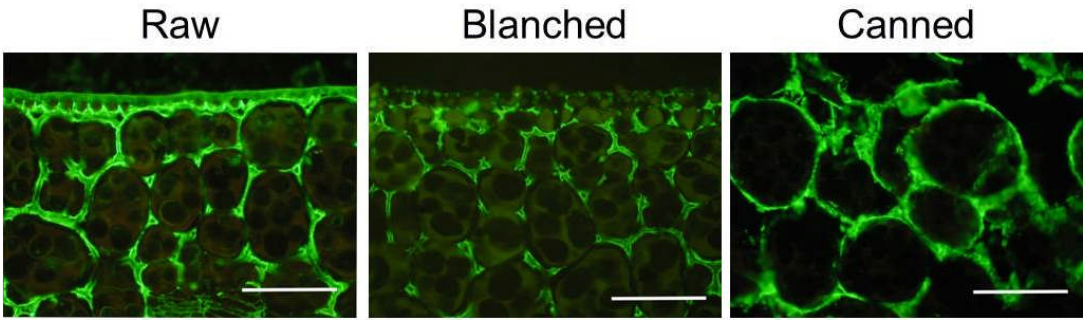

Figure 4.15 Respresentative micrographs of cotyledon sections of bean after different processing (raw soaked in formalyhyde, blanched and canned), indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5, with pre-treatment of Toluidine blue.

Showing the un-esterified HG changes of the beans after different processing. Scale bar for all micrographs = 100µm.

4.3.3.3 Effect of thermal processing on the other component in cell wall of the cotyledon

As mentioned in 4.2.1.4, LM1 binds extensin in the cell walls and LM21 binds with heteromannan in the cell walls. Figure 4.16 shows raw bean and canned bean binding with antibodies LM1, LM21. Small amount of extensin was found in both raw bean and canned beans. After canning, more extensin in the outer layer of cell wall was detected. This may cause by hydration degree which would provide better access for antibody to bind with the protein. LM21 epitopes were not detected for raw bean or canned beans. Marcus reported that LM21 epitopes was readily detected across the legume seed cotyledon after pre-treatment with pectate lyase (Marcus et al., 2010). The LM21 recognition can be revealed by enzymatic removal of pectic HG (Marcus et al., 2010), hence, the non-detection of LM21 epitopes was possibly blocked by the abundant HG in beans.

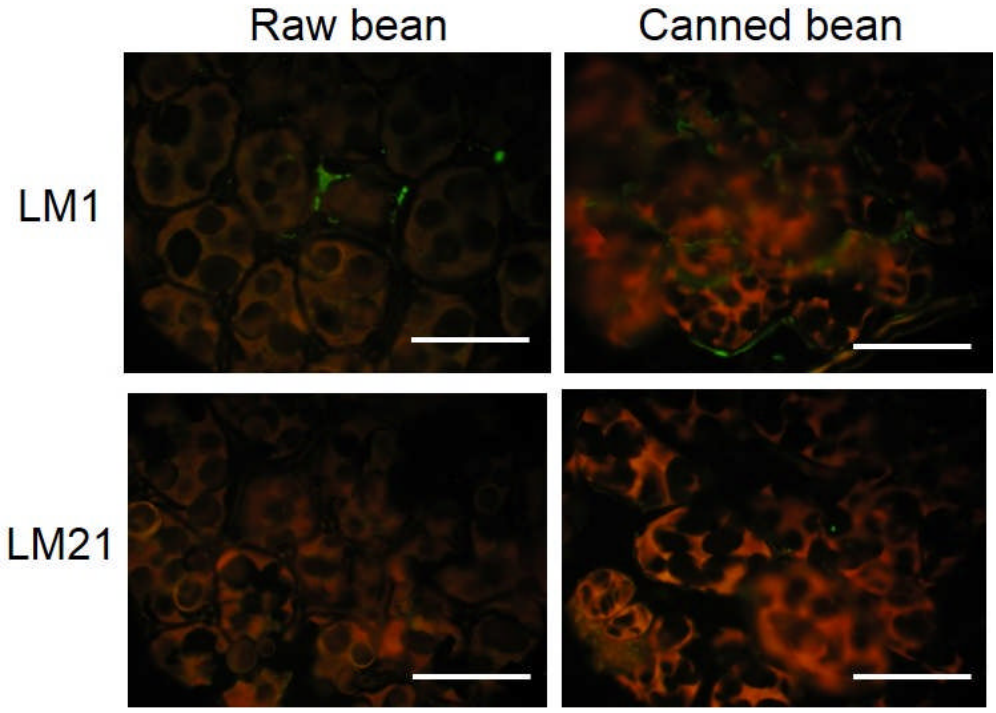

Figure 4.16 Respresentative micrographs of skin sections of raw bean soaked in formalyhyde, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM1 and LM21, with pre-treatment of Toluidine blue.

Showing the location of extensin and heteromannan respectivly. Scale bar for all micrographs = 100µm.

4.3.3.4 Effect of processing medium on the organization of beans

Different cooking medium was found to significantly affect the final canning quality including bean texture, drained weight and calcium levels (Balasubramanian et al., 2000). To investigate the effect of processing medium on the organization of bean, bean processed in different processing medium were smashed and labelled with antibodies.

Dry beans were ruptured using blender, canned beans cotyledons were scraped by hands. From Figure 4.17 fracturing raw beans ruptured cell walls and exposing the cell contents, the non-fracturing cells were still adhering tightly to each other, raw bean cells are hexagonal (Figure 4.17 a1). Pectin diffused everywhere, no labelling of cells could be observed (Figure 4.17 a2, a3, a4). All canned bean cells maintained their cell shape, separated readily along the surfaces of individual intact cell walls (Figure 4.17 b, c, d). The intercellular matrix of the middle lamella was loosened by the heat treatment, allowing the separation of cells without rupture of the cell walls

upon force (scraped by hand). Cell size increased up to 40% of original size after canning, the shape changed from hexagon to round or oval. For the canned beans in different medium, no obvious differences in cell size and shape were found. Pectin detection was brighter when canned in tomato sauce but no apparent difference for water and brine Figure 4.17 d4, d5). Figure 4.18 shows images from higher magnification observations. After canning the brighter binding may be the result of phenolic in the beans.

Rockland and Jones (1974) studied the cooked, water and salt-soaked beans, and found out no apparent differences between them in terms of the cellular structures. But different cooking rate appear to be related primary to differential rate at which cells separate, quick-cooking (salt-treated) increased the cell separation rate than normal cooking (Rockland and Jones, 1974). Therefore, it suggested that different cooking medium will not affect the cellular structure significantly, however, it affected the intercellular matrix by affect the cell adhesion.

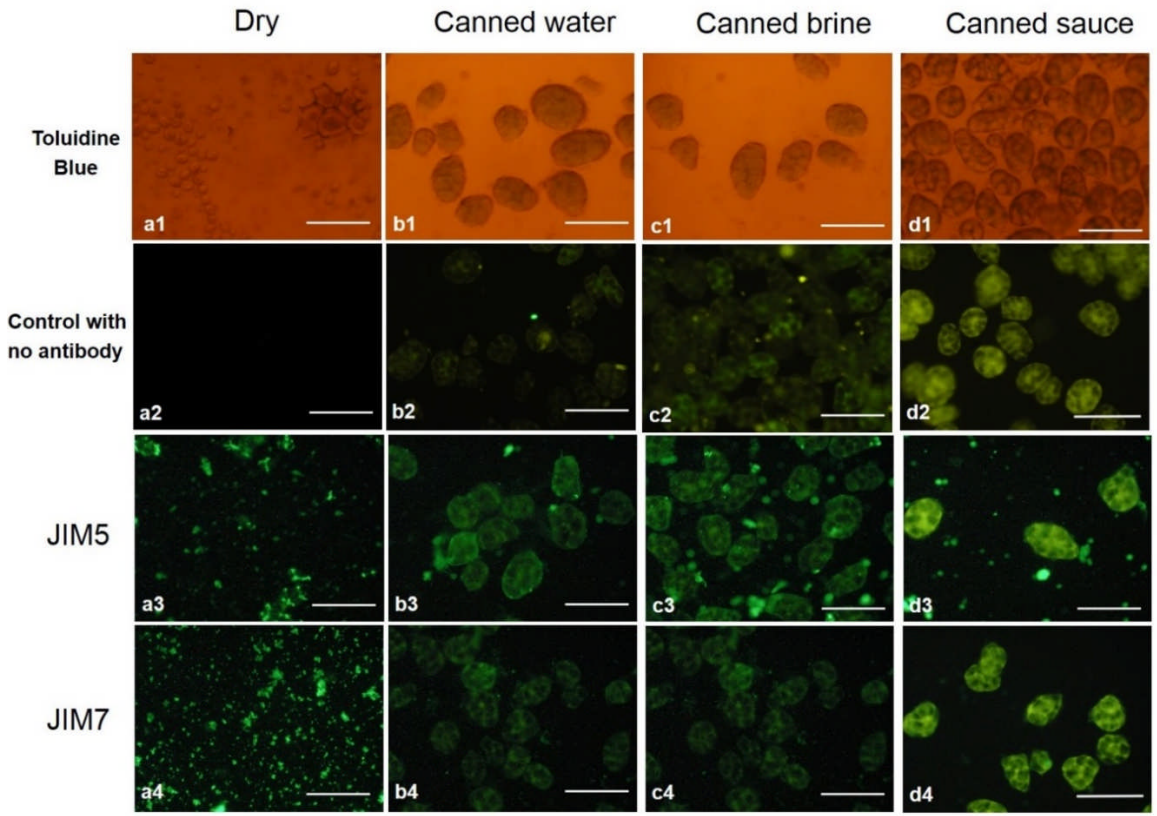

Figure 4.17 Effect of cooking medium on cell structure in navy bean cotyledon using different detection probe under 10× magnification.

Raw beans were ruptured using blender. Canned cooked beans cotyledons which cooked in water, brine and sauce were scraped by hands. Toluidine Blue stains the cell granules; JIM5 binding shows the un-esterified HG in the cell; JIM7 binding shows the esterified HG in the cell. Scale bar for all micrographs = 100μm

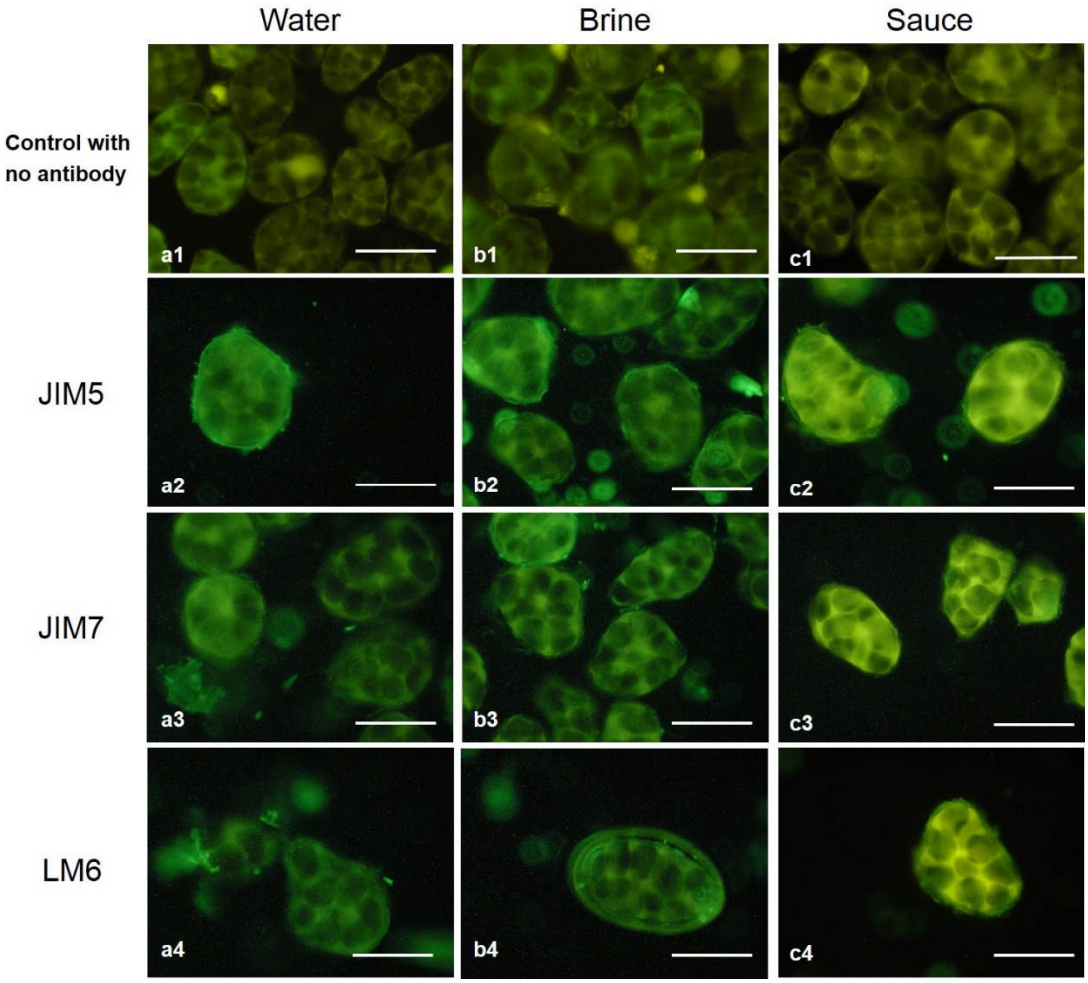

Figure 4.18 Effect of cooking medium on polysaccharides in navy bean cotyledon using different antibodies under 20× magnification.

JIM5 binding shows the un-esterified HG in the cell; JIM7 binding shows the esterified HG in the cell; LM6 binding shows the arabinan in the cell. Scale bar for all micrographs = 100µm

4.3.4 Effect of cultivar and region on the localization of cell wall polysaccharides in the cotyledon of raw beans.

In order to investigate the effect of cultivar and region on the cellulose in the cell wall, cotyledons were made into sections and stained with Calcofluor white. The firmest and softest cotyledon were selected. A1 which had the softest cotyledon had very thin cell walls, less cellulose staining. In constract, C2 which had the firmest cotyledon had very thick cell walls with much more cellulose staining (Figure 4.19). Therefore, tougher beans had thicker cell walls and presented more cellulose in the cotyledon cell walls. However, no obvious pattern can be found for the region or cultivar effect on the cellulose for eight types of beans without quantitative measuremnt.

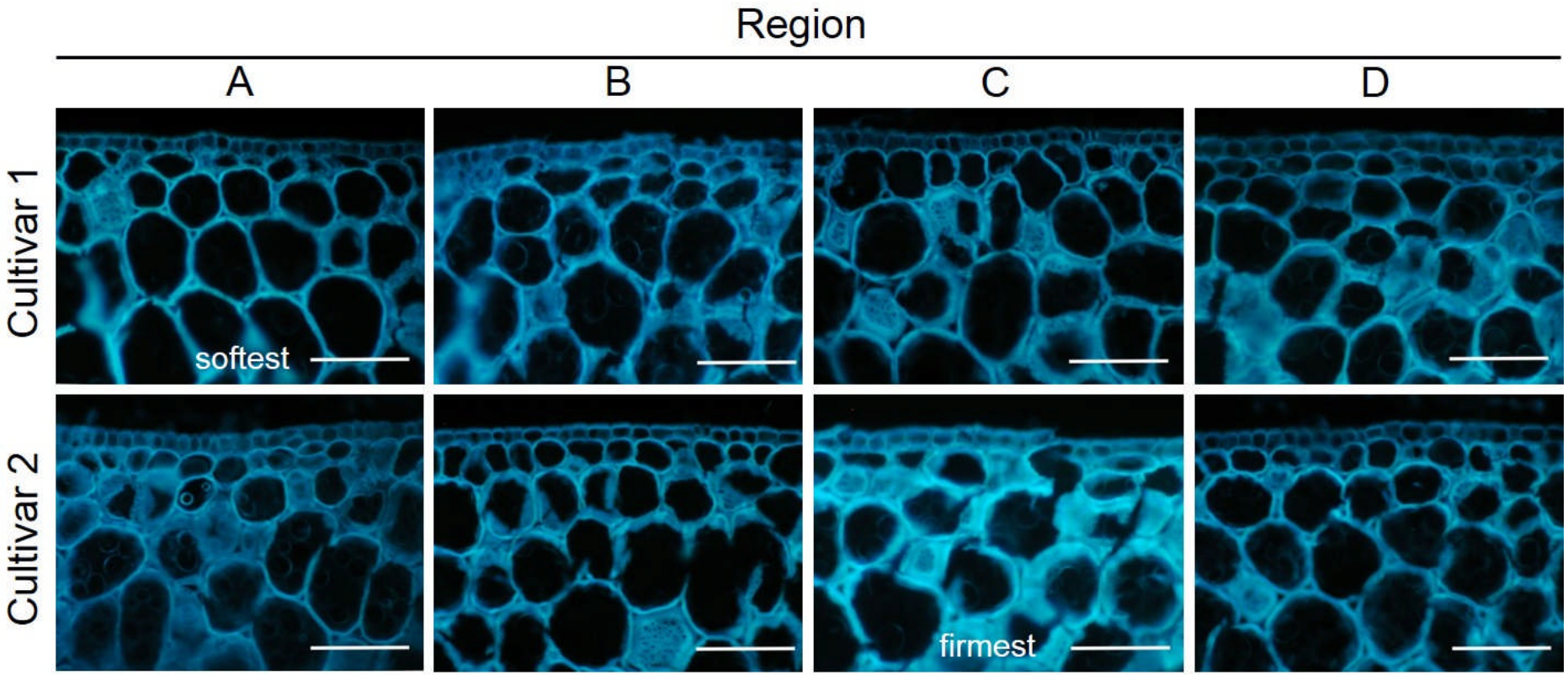

Figure 4.19 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde stained with Calcofluor white, from two cultivars and four regions.

Showing the location of cellulose in the cotyledon. Scale bar for all micrographs = 100µm.

In order to investigate the effect of cultivar and region on the localization of HG in cell wall, cotyledons were made into microscopies and labelling with antibodies JIM5 and JIM7.

Figure 4.20, Figure 4.21 shows the labelling of un-methyl-esterified HG and methyl-esterified HG in the cotyledon cell walls of raw navy bean. Un-methyl-esterified HG was abundant present in all beans, but no obvious pattern can be found for cultivar and region effect. C2 (the firmest cotyledon) presented less JIM5 epitopes binding than A1 (the softest cotyledon) (Figure 4.20), this can be explained as: the beans were soaked in formaldehyde directly without any processing, so that the beans were not hydrated enough to allow the water go through the intercellular area for the antibodies to bind. For the firm bean C2, the cells adhesion were very tight so that water was harder to go into the intercellular area, hence it was harder for the antibodies to bind. For the soft bean A1, the cells adhesion was looser than C2, so that it was easier for the water to go through and provided more surroundings for the antibodies to bind compared to C2. JIM7 epitopes binding were present less than JIM5 epitopes binding macroscopically (Figure 4.21). Beans that had more JIM5 binding appeared have less JIM7 binding (e.g. bean A1) because of the esterification degree (un-methylesterified HG was demethylesterified from methylesterified HG by pectin methylesterase (PME)). Same as un-methyl-esterified HG, no obvious pattern can be found for cultivar and region effect on the localization of methylesterified HG in cell wall.

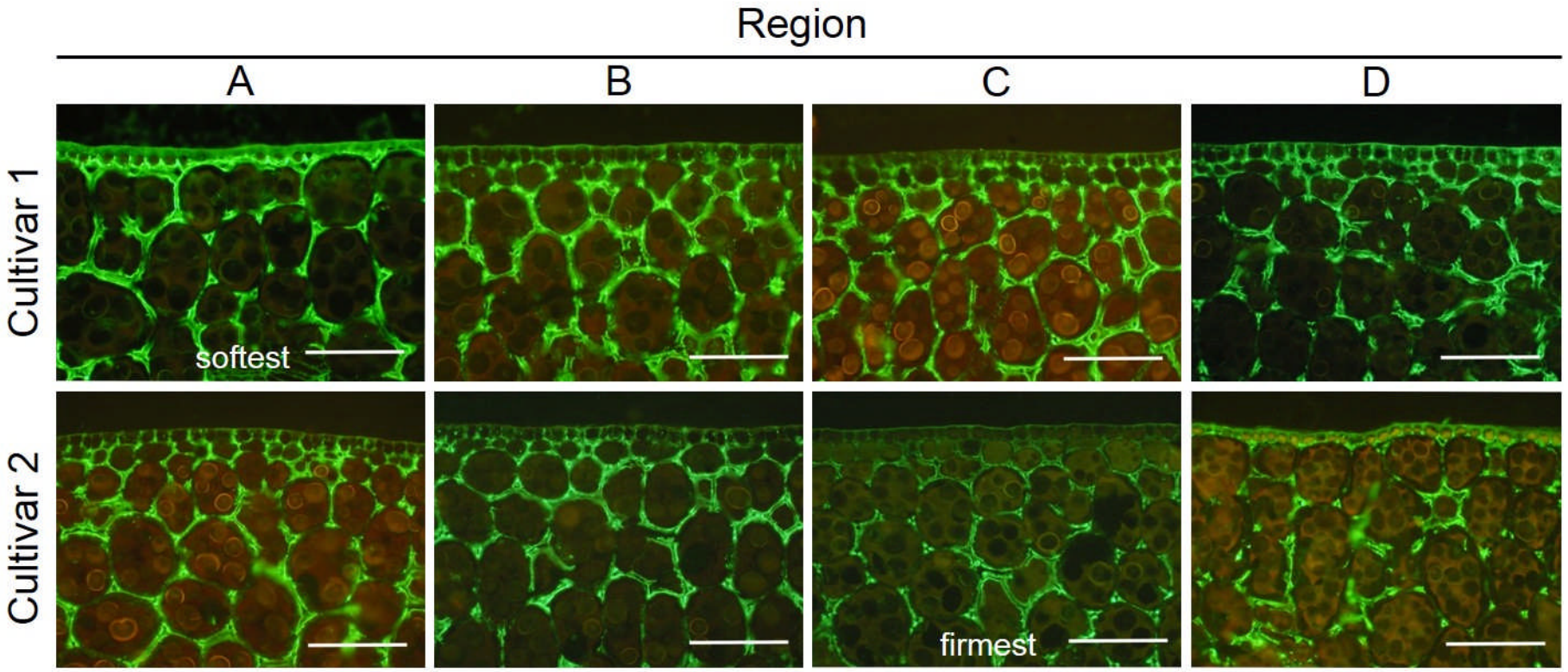

Figure 4.20 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5, with pre-treatment of Toluidine blue.

Showing the location of un-methyl-esterified HG. Scale bar for all micrographs = 100µm

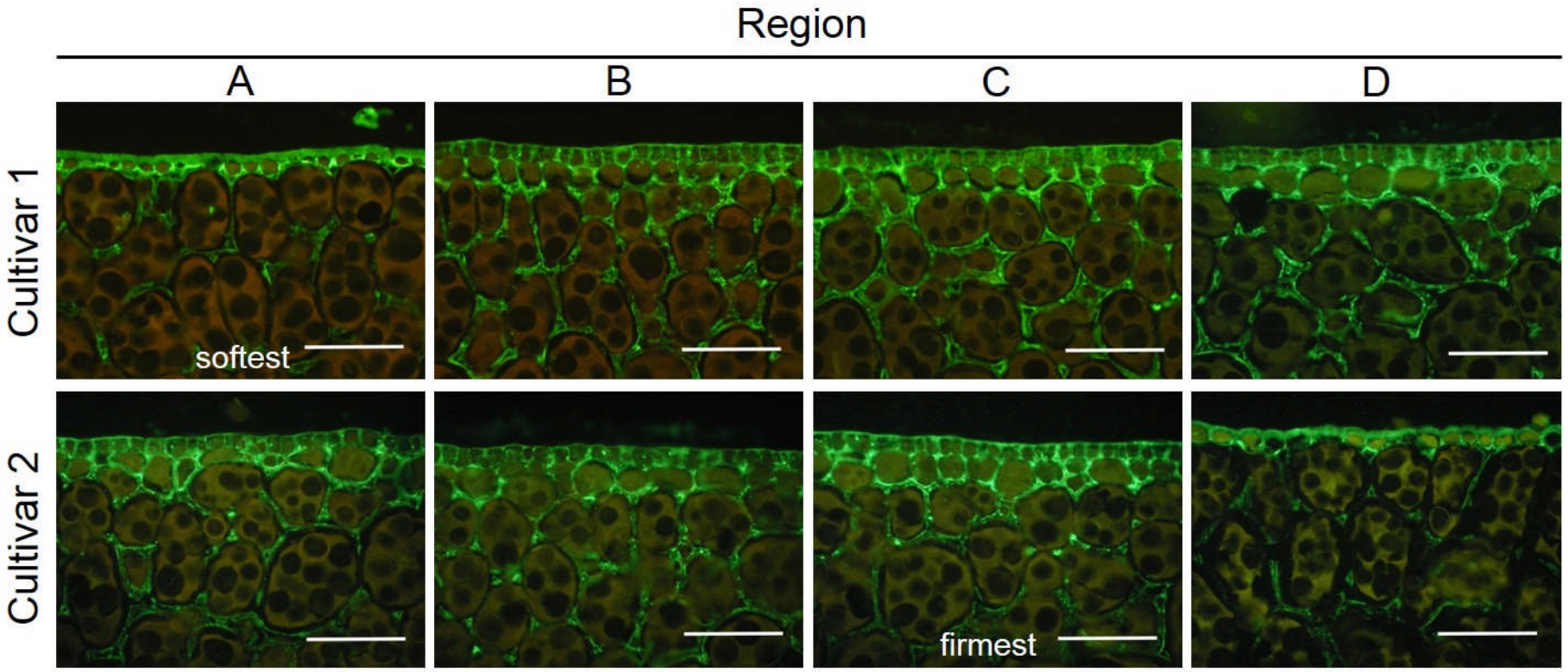

Figure 4.21 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM7, with pre-treatment of Toluidine blue.

Showing the location of methyl-esterified HG. Scale bar for all micrographs = 100µm.

In order to investigate the effect of cultivar and region on the localization of (1→4)-b-D-galactan and (1→5)-a-L-arabinan in cell wall, cotyledons were made into microscopies and labelling with antibodies LM5 and LM6.

Figure 4.22, Figure 4.23shows the labelling of arabinan and galactan in the cotyledon cell walls of raw navy bean. Region A presented more arabinan (Figure 4.22) and galactan (Figure 4.23) than the other regions, especially A1 which was the softest bean had the most abundant arabinan and galactan. As discussed above, this may be caused by the deficient hydration of the cells, so that fully hydration beans need to be discussed to conclude how the cultivar and regions affect the localization of pectic polysaccharides in the cell walls. Hence, canned beans are discussing below in the next section.

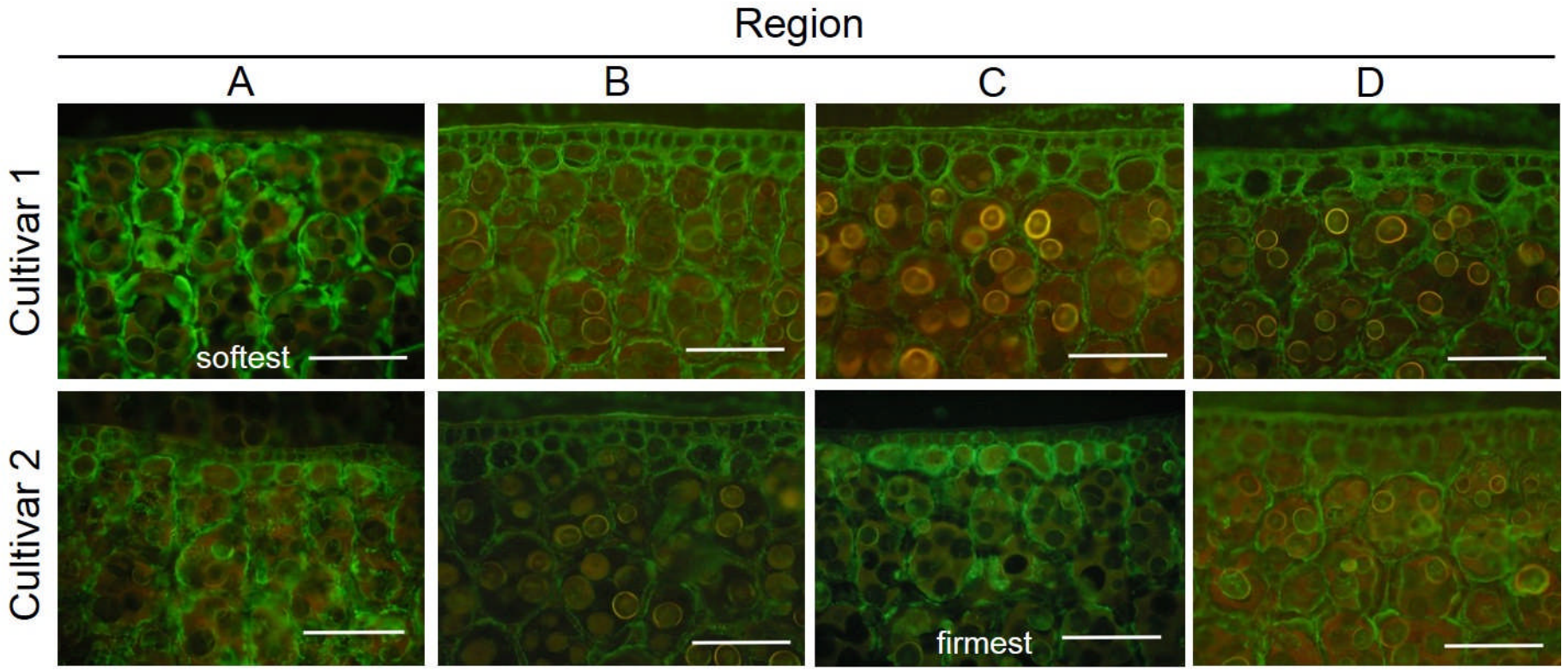

Figure 4.22 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM6, with pre-treatment of Toluidine blue.

Showing the location of (1→5)-a-L-arabinan. Scale bar for all micrographs = 100µm

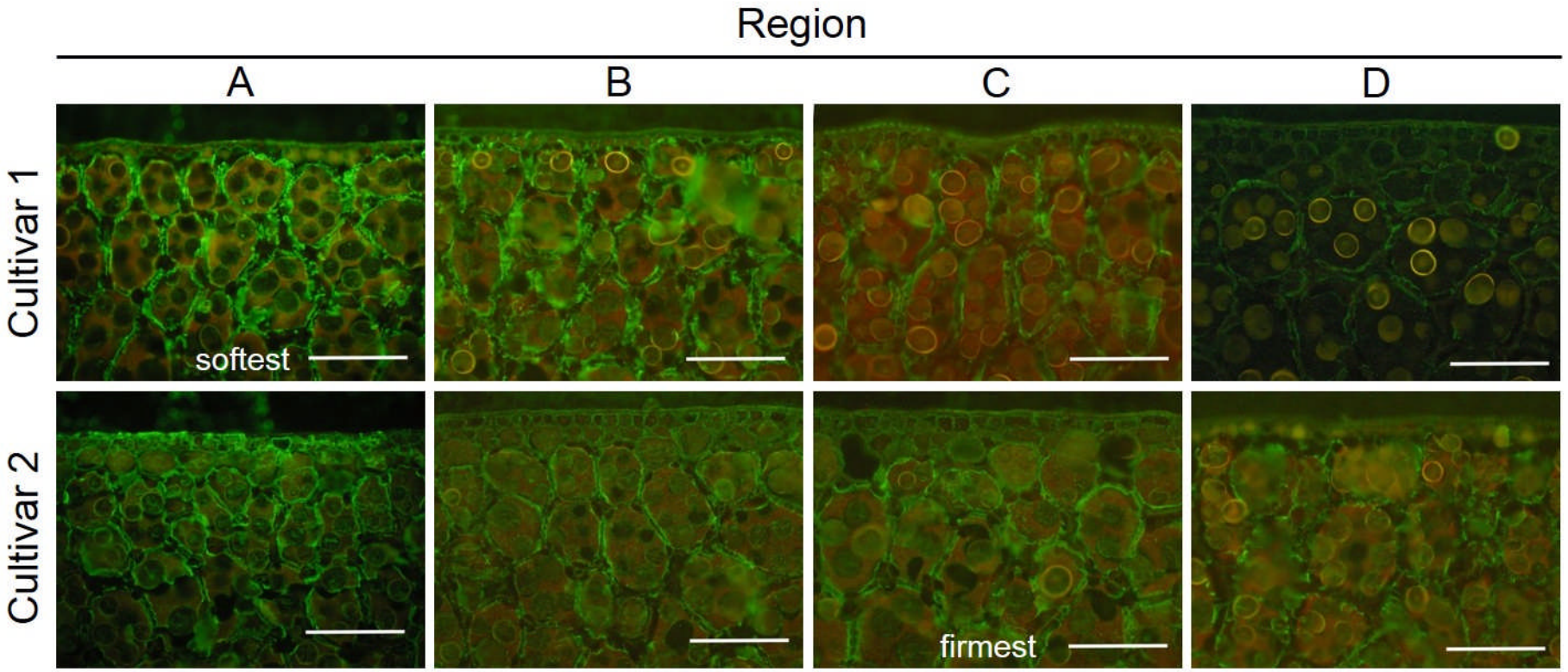

Figure 4.23 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM5, with pre-treatment of Toluidine blue.

Showing the location of (1→4)-b-D-galactan. Scale bar for all micrographs = 100µm.

4.3.5 Effect of cultivar and region on the localization of pectic polysaccharides in the cotyledon of canned beans.

In order to investigate the cultivar and region effect on the localization of pectin in cell wall for the canned beans, cotyledons of canned beans were sectioned and labelled with antibodies.

As discussed above, the hydration level may influence the bindings, and made it unclear to conclude the effect of cultivar and region. So the canned beans which were fully hydrated were studied. Figure 4.24 shows the labelling of un-methylesterified HG in the canned beans cotyledon. JIM5 epitopes were highly abundant present in the cell walls of all the beans. After canning process, the beans cells were swollen, cell adhesion was looser than blanched beans, middle lamella loosened significantly for all the beans except C1. Intercellular spaces triangle shape bindings were lost for most cells, only few were detected (arrows in Figure 4.24). Pectins surround the cells, middle lamella was filled with pectin as well. C1, which was considered as a firm bean, cells were tightly bound to each other and middle lamella did not loosen loosely. In C1, cells had smaller size than the other beans. The labelling of un-methylesterified HG can be clearly seen in the primary wall, middle lamella and intercellular spaces. Middle lamella was filled with more un-methylesterified HG than blanched beans, because beans were fully hydrated, middle lamella loosened significantly after canning which allowed better binding surrounding for the antibodies. Therefore, firmer beans seemed have a stronger cell adhesion contribute by un-methylesterified HG, the pectin adhered cells together to remain the rigidity of the cells. This adhesion prevent the cells to swell from absorbing the water into the cells, so it may indirectly decreased the water capacity of the beans.

Figure 4.25 shows the labelling of methyl-esterified HG in the canned beans cotyledon. JIM7 epitopes were less present than JIM5 epitopes, beans with stronger binding of JIM5 would have lighter binding of JIM7. For instance, bean B2 and C1, B2 had a very strong binding of JIM5 (Figure 4.24) but had a weak binding of JIM7 (Figure 4.25); C1 which had a very strong binding of JIM5 had a weak binding of JIM7. This is because the un-methyl-esterified HG was demethylesterified from the methyl-esterified HG by the pectin methylesterases (PME). So the beans that had

more un-methyl-esterified HG would have less methyl-esterified HG. No obvious pattern was found for the cultivars and regions effect.

Figure 4.26 shows the labelling of arabinan in the canned beans cotyledon. Compared to raw beans, arabinan labelling was also much more abundant present in the cell walls because of the hydration of cells. Different from JIM5 and JIM7, LM6 epitopes were throughout the primary walls but more abundant in the inner region of the primary cell wall close to the plasma membrane. Similar as JIM5 and JIM7, LM6 epitopes binding cannot see distinct difference between beans either, no obvious patterns were found for the cultivar or region effect.

No reports have studied how cultivar and region effect on the microstructure of the beans. From the direct visible results (microscopies), it is suggested that under fully hydration conditions, cultivar and region were not found to have effect on the labelling of pectic polysaccharides in the cell wall as investigated by immunofluorescence microscopy. However, firm beans tended to have more pectin in the intercellular area and less loosened cell lamella.

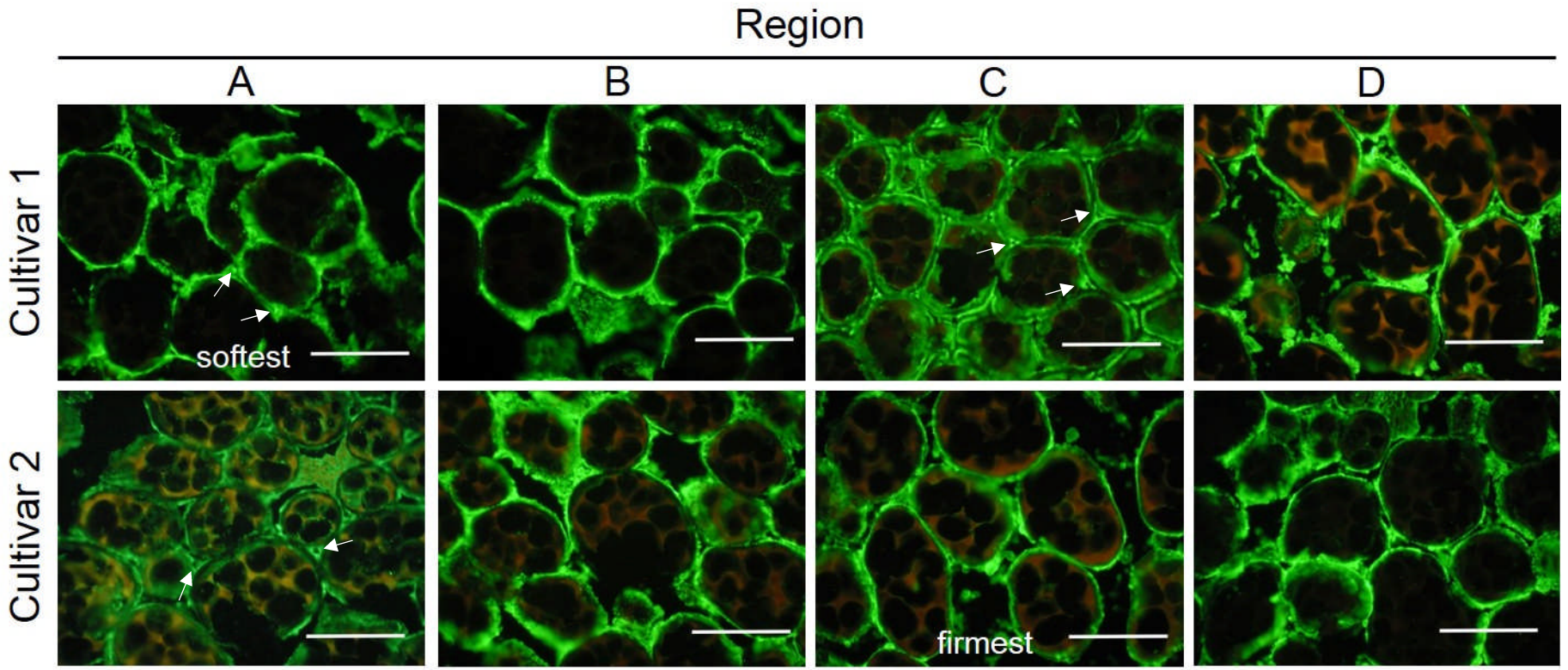

Figure 4.24 Respresentative micrographs of cotyledon sections of canned bean from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5, with pre-treatment of Toluidine blue.

Showing the location of un-methyl-esterified HG. Arrows points to the intercellular spaces binding. Scale bar for all micrographs = 100µm

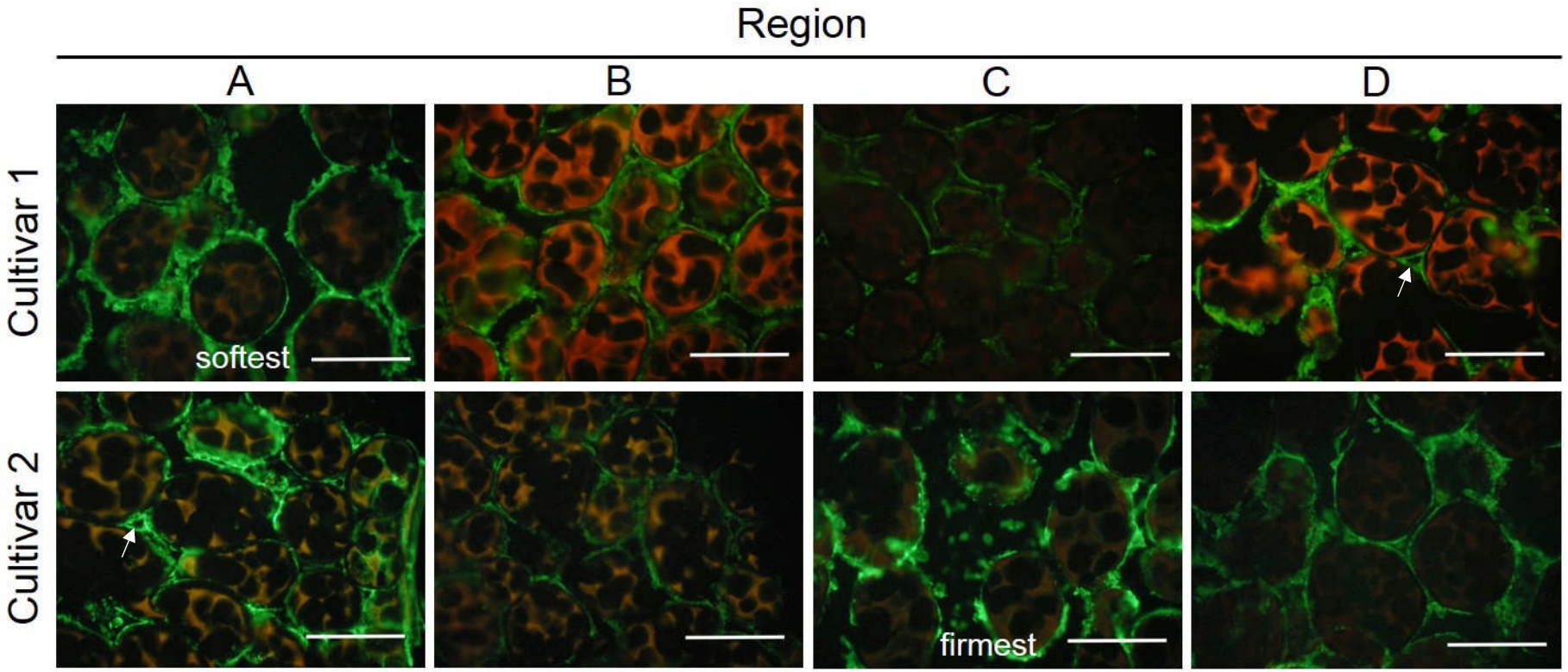

Figure 4.25 Respresentative micrographs of cotyledon sections of canned bean from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM7, with pre-treatment of Toluidine blue.

Showing the location of esterified HG. Arrows points to the intercellular spaces binding. Scale bar for all micrographs = 100µm

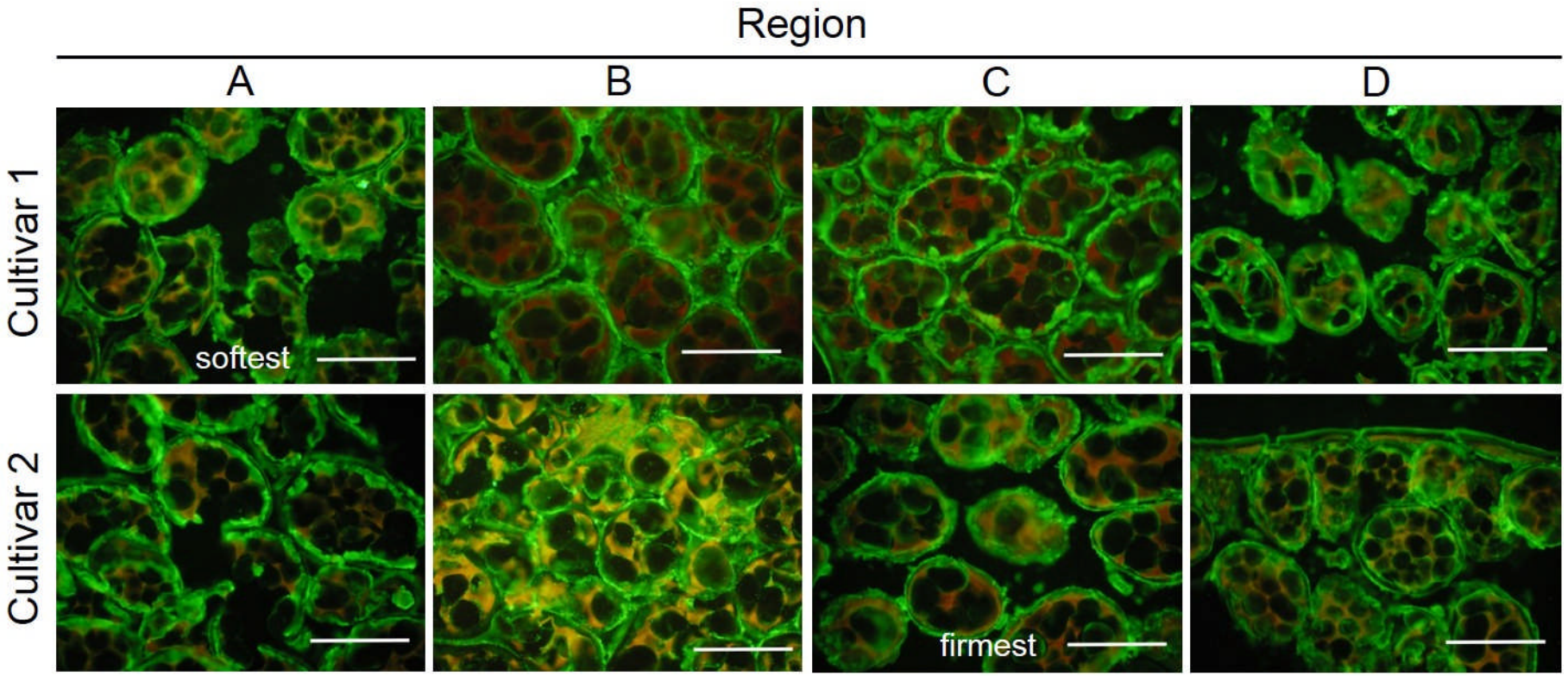

Figure 4.26. Respresentative micrographs of cotyledon sections of canned bean from two cultivars and four regions, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody LM6, with pre-treatment of Toluidine blue.

Showing the location of (1→5)-a-L-arabinan. Scale bar for all micrographs = 100µ

4.3.6 Localization of pectic polysaccharides for the other varieties beans.

Eight types of beans were selected to study the cultivar and region effect. Besides those, 9 other varieties also have been studied. According to Chapter 3, C5, C4 was relatively soft compared to the other beans and Ch09 and Ch10 were relatively firm compared to the other beans. Figure 4.27 shows the un-methyl-esterified HG in the other varieties besides the eight types. Firm beans (Ch09 and Ch10) only bind brightly in the intercellular spaces. However soft beans (C5 and C4) seem to have more binding in the middle lamella than firm beans. It may because firm beans cells adhered to each other more tightly that it allows less water to come into the middle lamella, so that the antibody did not have enough substance to binds with pectin. Therefore, from the other varieties beans, it also indicated that firmer beans had a tighter cell adhesion, which obstruct the water coming between the cells and caused less binding.

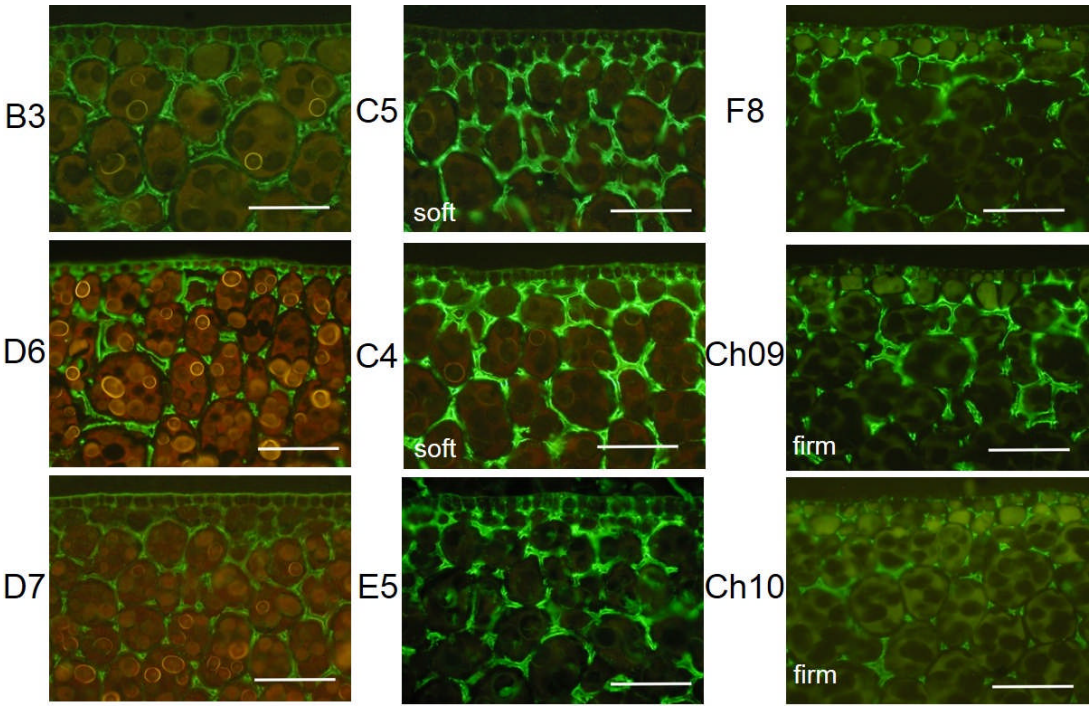

Figure 4.27 Respresentative micrographs of cotyledon sections of raw bean soaked in formalyhyde, indirect immunofluorescence microscopy of sections labelled with monoclonal antibody JIM5, with pre-treatment of Toluidine blue.

Showing the location of un-esterified HG. Scale bar for all micrographs = 100µm

4.4 Conclusion and discussion

Beans structure and microstructure characteristics were discussed in this chapter. The skin of navy beans was found to be rich in cellulose, so it provided a tough obstacle to the outside environment, particularly exchange of water and solutes. Raw navy beans skin was found to be abundant in pectin but most of the pectin was lost during the processing, and this may explain why a lot of skins are lost during canning. Although skin plays an important role in the beans, the major determinant for the sensory response to canned beans is cotyledon firmness, therefore cotyledon microstructure is focus to discuss in this project.

Cotyledons which had a firmer texture were found to have a tighter cell adhesion, so that it may have allowed less water go in between cells when blanching, and cell separation was less likely to occur when canning. Cotyledon cell wall was abundant in pectins (including HG, galactan and arabinan). The most abundant polysaccharide was found to be un-methylesterified HG, it was found to be present in the primary wall, middle lamella and intercellular spaces. After canning, cells appeared to swell up, increasing in volume and becoming rounder, however the cells were not broken. Un-methylesterified HG was still abundant in primary wall and middle lamella. Beans that had more un-methylesterified HG were found to have less methylesterified HG (JIM7 epitopes), indicating the likely activity of pectin methylesterase (PME). LM5 epitopes (galactan) and LM6 epitopes (arabinan) was less present than JIM5 epitopes. Galactan was found to be present in restricted locations at the inner region of the primary wall close to the plasma membrane, and arabinan was found to present throughout the primary wall and more present in the inner region of primary wall close to the plasma membrane. Both galactan and arabinan were not rich in middle lamella or intercellular spaces. Extensin and mannan were not found abundantly present in navy beans cell wall. As hemicellulose is generally abundant in cell wall, LM15 probe can be used in the future to detect the hemicellulose xyloglucan in the navy bean (Marcus et al., 2008).

Cell adhesion is largely attributed to the properties pectin, most particularly calcium cross linked homogalacturonan (Jarvis et al., 2003, Mohnen, 2008, Orfila, 2001). During the canning process of vegetables, solubilisation and depolymerisation of pectin occurs because of the thermal treatment in water (Brett and Waldron, 1996). If pectin from the middle lamella is largely lost then it would cause cell separation.

In the canned navy beans, soluble pectin was detected in the cooking medium using ELISA in chapter 6, the middle lamella appeared to have a looser appearance. However, there must still be enough pectin in the middle lamella to keep cells adherent because the beans are intact after canning. However, upon pressure (e.g. compression or shear) being applied on the beans, the cell adhesion forces are not enough to keep the cell together and breaking of the tissue occurs between cells rather than through cells. This phenomenon is associated with a soft but sandy texture. Similar observations were made in cowpea and lima beans, where fracture occurred in the middle lamella leaving most of the cells intact (Rockland and Jones, 1974, Sefadedeh et al., 1978). Therefore, firm beans were found have tighter cell adhesion than soft beans, but all the beans cells were intact after canning. It is thought that a major contribution to intercellular adhesion comes from the chelator-soluble pectin rich in acidic homogalacturonan (Walter and Taylor, 1991), so it is possible that intercellular homogalacturonan depolymerised due to the harsh processing during canning but was not solubilised due to physical entrapment, but also due to the presence of tomato pectin and starch in the sauce which decrease the osmotic potential. HG was believed to contribute to intercellular cell adhesion. Un-methylesterified HG which cross links to calcium would form gel and contribute to the cell adhesion and consequently contribute to the firmness of the beans. Although the functionality of the side chains of RGI is not yet clear (Orfila and Knox, 2000), they have been suggested to contribute to cell adhesion (Caffall and Mohnen, 2009), and their presence is highly dependent on the development stages of the plant tissue (Orfila and Knox, 2000, McCartney et al., 2000). Hence, although galactan and arabinan may contribute to the cell adhesion in navy beans, their function need to be investigate further in different development stages of beans to obtain a solid conclusion.

The effect of cultivar and region on the localization of cell wall components (including cellulose and pectin) was not conclusive from the microscopies. It is the first time that antibody techniques are used to localise the cell wall polysaccharides in navy beans. Antibodies successfully localised the pectins in the cell wall by using immunofluorescence microscopy, and for the first time visualised the cell wall structure of the navy beans. The microscopies gave direct visual image about the change of the cell wall structure during processing, although they were not able to recognise the subtle differences between cultivar and region. Antibodies are very

specific probes that recognise specific structural features of pectin that are exposed at the surface of the section. Hence, antibodies only bind to the exposed pectins in the beans, the pectins embedded into the cell wall cannot be bound by the antibodies, because antibodies are large proteins and not able to assess into the cell wall to bind the pectin. This masking effect has been described by Knox and co-workers, who unmasked cell wall epitopes in sections treated with chemical reagents or enzymes (Marcus et al., 2010, Verhertbruggen et al., 2009).

As mechanical properties were found to differ between cultivars and regions, it is possible that immunocytochemical method has limitation for quantification analysis. Therefore, quantitative method are needed to quantify the composition of the cell wall components to investigate the effect of cultivar and region. In the next chapter, the chemical composition of beans will be investigated.

Chapter 5
Carbohydrate composition of canned baked beans

5.1 Introduction

The microstructure of the cell wall changed and cell adhesion loosened significantly after canning, as observed using immunolocalization microscopy presented in chapter 4. Thermal processing caused cell wall swelling and loosened middle lamella. However, the pectic epitopes were still abundantly present in walls and microscopy is not quantitative and cannot observe the effect of cultivar and region. Therefore, in this chapter, the carbohydrate compositions of canned beans were determined using analytical techniques, and the effect of growth region, cultivar, processing on the carbohydrate of canned beans was investigated.

In this chapter, the aims were: 1) to determine the amount of the dietary fibre including water insoluble polysaccharides (WIP) and water soluble polysaccharide (WSP); 2) to determine the amount of the starch content; 3) to determine the composition of WIP and WSP after hydrolysing them into monosaccharides; 3) to investigate the effect of growth region and cultivar on the content and composition of carbohydrates in canned baked beans (WIP, WSP and starch).

The dietary fibre of the canned beans was isolated following enzymatic digestion of starch and protein, and recovery of the fibre residue. Dietary fibre can be separated into water insoluble polysaccharides (WIP) and water soluble polysaccharide (WSP) after alcohol precipitation. WIP and WSP were then hydrolysed to monosaccharides by 2M TFA and their composition was determined by HPAEC-PAD. The starch of the canned beans was digested by enzymes α-amylase and amyloglucosidase. The amount of glucose released was determined by HPAEC-PAD and converted to starch content.

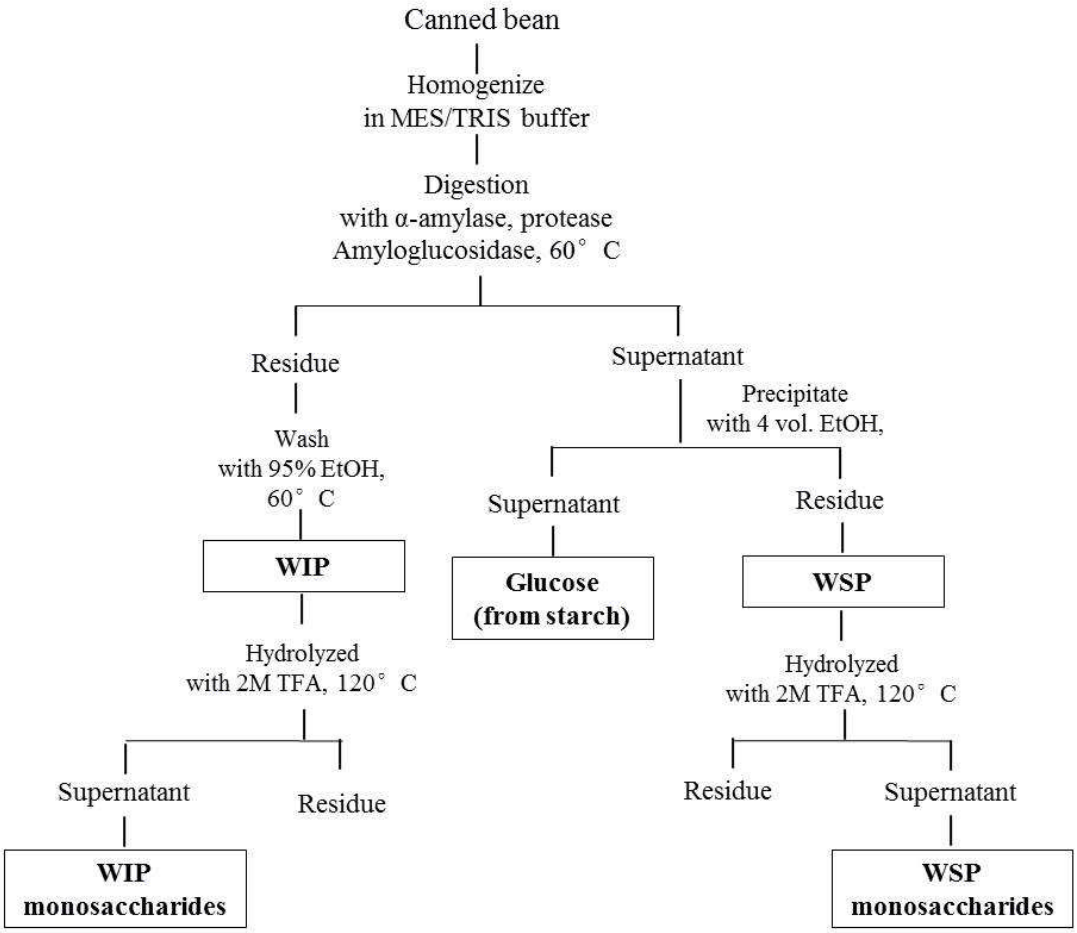

Figure 5.1. General flow chart of dietary fibre isolation procedure; extraction of the water insoluble polysaccharides (WIP), water soluble polysaccharide (WSP) and glucose from starch.

5.2 Effect of cultivar and region on the dietary fibre content of canned beans

Dietary fibre includes non-starch polysaccharides and resistant starch. The non-starch polysaccharides come from cell wall (Table 1.2). Dietary fibre can be extracted as water soluble polysaccharides (WSP) and water insoluble polysaccharides (WIP). Figure 5.2 shows the picture of dried extracted WIP and WSP of canned baked beans. WIP is white colour, with a loose crisp texture. WSP is amber colour, with a toffee sugar texture.

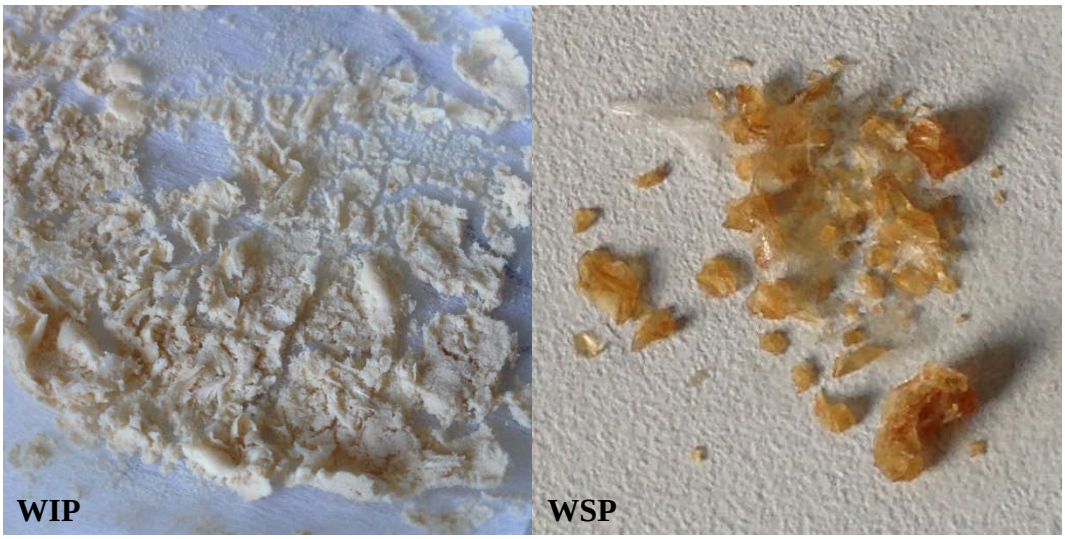

Figure 5.2. Image of WIP and WSP.

Table 5.1 shows the dietary fibre content (including WIP and WSP) of canned baked beans. Canned beans contained 10.8% - 16.4% (w/w %, wet weight basis) of dietary fibre, 9.2% - 14.8% (w/w %, wet weight basis) is WIP and 0.7% - 1.6% (w/w %, wet weight basis) is WSP. WIP contributed 85% - 95% of (w/w %) and WSP contributed 5% - 15% (w/w %) of the weight of dietary fibre. Canned beans contained 63% of water. So when calculated as dry weight basis, they contained 30.6% - 42.2% (w/w, dry weight basis) dietary fibre in canned beans, including 26.2% - 38.2% (dry weight basis) WIP and 2% - 4.5% (dry weight basis) WSP.

In order to investigate the effect of cultivar and region on the dietary fibre content of the beans, two-factor ANOVA was carried out (Table 5.2). The dietary fibre content was found to be significantly affected by growth region and cultivar. Beans from region B (14.1%) and C (15.7%) had higher amount of dietary fibre than A (11.8%) and D (12.1%). Cultivar 2 had significant higher amount of dietary fibre than

cultivar 1. For WIP, cultivar and region were found to have effect on the WIP content with interaction, beans from region C (14.2%) had significantly higher amount of WIP compared to the other beans except B2 (Table 5.1). Beans from region D had significant lower level of WIP. For WSP, cultivar and region did not significantly affect the amount of WSP because of the small value and big variance of the data (31% variation, ratio of the standard deviation to the mean).

Table 5.1. Amount of WIP, WSP and dietary fibre in 100g of canned navy beans (wet weight basis).

Bean name	WIP	WSP	Dietary fibre (WIP+WSP)
		(g/100g bean)	
A1	9.2 [a]	1.5	10.8[a]
A2	12.2 [b]	0.7	12.9[ab]
B1	11.7 [b]	1.6	13.4[ab]
B2	13.8 [bc]	1.1	14.9[abc]
C1	13.5 [bc]	1.5	15.0[bc]
C2	14.8 [c]	1.6	16.4[c]
D1	10.9 [a]	1.0	11.9[a]
D2	10.8 [a]	1.4	12.2[a]

Values show mean of 3 replicates. Letters a, b, c means within same column letters are not significantly different at $p<0.05$ levels.

There were no studies investigated the effect of cultivar or region on dietary fibre content of canned beans. But there were some studies that reported the effect of cultivar or region on dietary fibre content of raw beans. Cultivar was found to have significantly effect on the amount of WIP in seed coat, dietary fibre and WSP in cotyledon for raw beans. Cultivar C-20 had smallest amount of dietary fibre and

WSP in cotyledon, but highest amount of WIP in seed coat (Srisuma et al., 1991). Region was found to have no significant effect on WIP for raw beans (Kereliuk and Kozub, 1995). However, raw beans cell wall polysaccharides are not comparable to canned beans because the thermal treatment significantly changed the polymers constituent by degrading and solubilising the polymers (Stolle-Smits et al., 1995, Stolle-Smits et al., 2000).

Table 5.2. Two - factor ANOVA P-value for the effect of cultivar and region on WIP, WSP and dietary fibre for canned beans.

	WIP	WSP	Dietary fibre
Cultivar	<0.001	0.21	0.0031
Region	<0.001	0.44	<0.001
Cultivar : Region	0.039	0.070	0.40

At 95% confidence interval

Table 5.3 shows the comparison of the average value for compositions in cooked and raw navy beans in different reports. Aldwairji et al. (2014) studied the dietary fibre content of canned baked beans with tomato sauce and found canned beans (with tomato sauce) contained 16.1% (w/w %, dry weight basis) of dietary fibre, 9.1% (w/w %, dry weight basis) was WIP and 7% (w/w %, dry weight basis) was WSP. The much lower dietary fibre content in Aldwairji's project may have two explanations: first, they studied the baked beans fibre content with the tomato sauce. It had a very low ratio of WIP to WSP, this indicated high amount of WSP was from tomato sauce because tomato was rich in pectic polysaccharides (Orfila et al., 2001). Second, the samples they used were mix samples of 5 brands of canned baked beans. Samples variation would significantly influence the fibre content. Heinz baked beans are famous with its intact beans while other brands have more smash beans.

Costa et al. (2006) investigated dietary fibre content of beans soaked and boiled in water, and claimed that cooked common beans (in water) contained 25% (w/w %, dry weight basis) of dietary fibre, including 22.6% (w/w %, dry weight basis) of WIP and 2.6% (w/w %, dry weight basis) of WSP. Harvard (2004) reported cooked beans (in water) contained 21.3 % (w/w %, dry weight basis) dietary fibre, including

15.4% (w/w %, dry weight basis) WIP and 5.9% (w/w %, dry weight basis) WSP. Significant higher amount of WIP was detected in this project compared to these two studies. It is possible that different processing would affect the dietary fibre content, also the different bean samples used may have variation in fibre content. However, when we took starch content into consideration, the carbohydrate content (dietary fibre + starch) in this project (64.3%) is similar to Costa et al. (60%). Therefore, it is possible that the dietary fibre content detected in this project contained some non-hydrolysed starch.

Raw bean composition was studied by Costa (2006) which claimed that raw common bean (navy bean is a class of common bean (phaseolus vulgaris)) contained 21.9% (w/w %, dry weight basis) WIP and 2.64% (w/w %, dry weight basis) WSP. Kereliuk (1995) claimed raw navy bean contained 17% - 19% (w/w %, dry weight basis) dietary fibre. Srisuma et al. (1991) and Shiga et al. (2006) studied the dietary fibre with separation of skin (seed coat) and cotyledon. Srisuma et al. claimed that the dietary fibre in skin contained 92% - 95% (w/w %) WIP and 2.4% - 3.8% (w/w %) WSP; dietary fibre in cotyledon contained 19.4% – 28.4% (w/w %) WSP, but they did not give the ratio of skin to cotyledon in the whole bean. Shiga et al. (2006)claimed that bean consisted of 10% (w/w %, dry weight basis) of skin and 89% (w/w %, dry weight basis) of cotyledon in a whole bean. Skin contained 7.3% (w/w %, dry weight basis) of dietary fibre of the whole bean, including 7% (w/w %, dry weight basis) of WIP and 0.3% (w/w %, dry weight basis) of WSP. Cotyledon contained 16.6% (w/w %, dry weight basis) dietary fibre of the whole bean, including 9.6% (w/w %, dry weight basis) WIP and 7% WSP (w/w %, dry weight basis).

Raw beans dietary fibre content tended to have lower amount compare to cooked beans. It is believed that thermal process changes the physico-chemical characteristics of legumes. Processing increases nutrient availability in legumes, such as dietary fibre, digestible starch content, protein and resistant starch (Costa et al., 2006, Rehman and Shah, 2005). For example, raw beans are not digestible for human, but after cooking, it becomes digestible for human. So that, processing made more dietary fibre be able for the enzyme to extract out. Costa (2006) found that both insoluble and soluble fibre of common bean (navy bean) significantly increased after cooking, and same situation happened to pea as well. It indicated cooking

allowed more insoluble and soluble fibre to be extracted by enzyme. Therefore, canned baked beans may detect higher percentage of dietary fibre compared to raw navy beans.

Variations for the composition amount detected among different projects also depended on the methods that being used and different bean samples. The method used to obtain the dietary fibre in this project is modified from (AOAC) (AOAC, 1995) official method (991.43) (Chapter 2. Section 2.2.8.2.). Aldwairji et al. (2014) and Costa et al.(2006) used the similar method as using in this project, which is using enzyme digestion to remove the starch (α-amylase, Termamyl) and protein (protease), measure the residue afterwards. Costa et al. used shorter incubation time for amylase (15min instead of 35min in this project and Aldwairji's project) and longer incubation time for the proteases (2h instead of 30min in this project and Aldwairjia's project). Kereliuk used different method to measure fibre content which is colorimetric method – sugars gave a colour change when treated with phenol-sulfuric acid. Besides the method influence, beans samples collected from different cultivars and regions would have significantly differences biologically; different enzyme used in digestion procedure may also significantly influence the results. Hence, it is necessary for the methodological standardization for obtaining better uniformity of the data (Costa et al., 2006).

Table 5.3. Comparison of the carbohydrate compositions (% w/w, dry weight basis) in navy beans from different literature[A]

		Reference		**Dietary fibre (WIP+WSP)**	WIP	WSP	Starch
Canning in tomato sauce	Without sauce	This project		**36.3**	32.2	4.1	28
	With sauce	(Aldwairji et al., 2014)		**16.1**	9.1	7.0	N/A
Soaked and boiled in water		(Costa et al., 2006)		**26.5**	23.8	2.7	33.5
		(Harvard University, 2004)		**21.3**	15.4	5.9	N/A
Raw		(Costa et al., 2006)		**24.8**	22.1	2.7	29.2
		(Kereliuk and Kozub, 1995)		**18**			36
		(Shiga and Lajolo, 2006)	Cotyledon	**16.6**	9.6	7	N/A
			Skin	**7.3**	7	0.3	N/A

A. Data was the average value adopted from different reports.

5.3 The composition of dietary fibre (WIP + WSP) for canned beans

Dietary fibre consists of WIP and WSP. The bulk of dietary fibre is composed of water insoluble polysaccharides (WIP). WIP is thought to contain cellulose, hemicellulose, pectic polysaccharides, resistant starch and small amount of lignin and ash. Cellulose presented predominantly in WIP at 48% (w/w %), followed by hemicellulose at 32% (w/w %) (Srisuma et al., 1991). Small portion of dietary fibre is water soluble polysaccharides (WSP) which contribute 19% - 28% (w/w %) of the dietary fibre (Srisuma et al., 1991). The main component for WSP is pectic polysaccharides, but can also contain hemicelluloses.

In order to analyse the composition of WIP and WSP, WIP and WSP were hydrolysed by heating in 2M TFA for 1h at 120°C. The supernatant was collected for determining the monosaccharides by HPAEC-PAD with a CarboPac™ PA20 column. Figure 5.3 shows standard separation of 9 cell wall monosaccharides in a concentration of 0.1µg/µl. The elution program has been optimised for two months until achieve this separation method. Monosaccharide standards, including L-Fucose, L–Rhamnose, L-Arabinose, D-Galactose, D-Glucose, D-Xylose, D-Mannose, D-Galacturonic acid and D-Glucuronic acid, were separated using the optimised method. Xylose and mannose came out in one peak, so another method is needed to separated xylose and mannose individually if required (data not available in this project). Cell wall sugars in the samples are very hard to hydrolyse, because cellulose-hemicellulose network is cross-linked and permeated by pectins. Hydrolysis can be affected by acid or enzymes (Fry, 1988), Fry recommended that for non-cellulose polysaccharides, neutral pyranosyl residue can be hydrolysed in 2M TFA at 120°C for 1 hour, however, under this condition, the acidic pyranosyl including glucuronic acid and galacturonic acid are incompletely hydrolysed with a low yield of monosaccharides, because the uronic acid glycosyl linkages are relatively acid resistant (Fry, 1988). Cellulose can be hydrolysed using 1M sulfuric acid.

Figure 5.4 shows the representative elution profile for the WIP and WSP from canned beans. The major differences between WIP and WSP profile were arabinose and glucose peak height. Glucose was the predominant component in WIP, and

arabinose was the predominant component in WSP. In Figure 5.4 A, WIP elution profile, peak 6 and 7 was considered as un-identified peak, it is possible they were uronic acid which were partly hydrolysed by TFA, however because of the elution time was shifted forward, it cannot confirm that they were uronic acid. The solution to identified these two peaks can be spiked by adding galacturonic acid and glucuronic acid, if the peak increase, it can confirm that they are the uronic acid. As observed in Figure 5.4 B, no uronic acid peaks were detected in WSP.

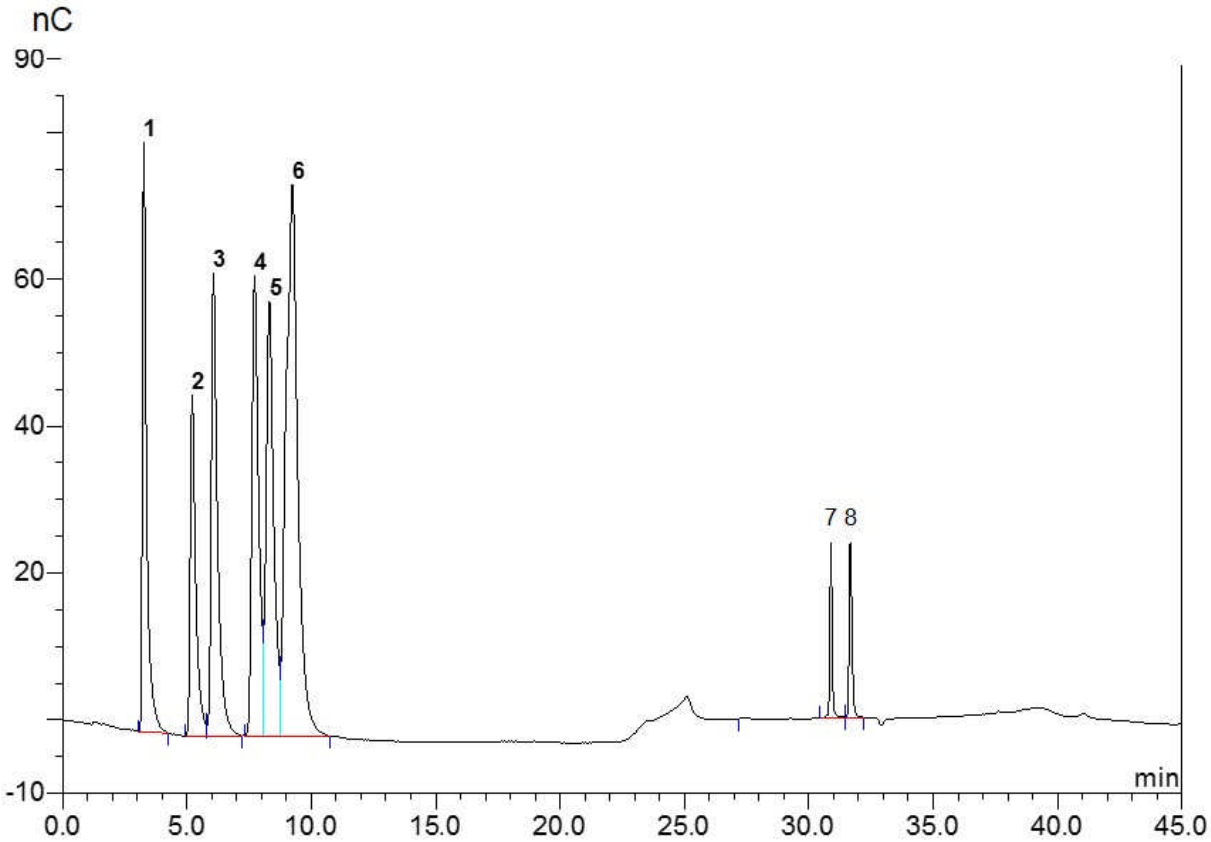

Figure 5.3. HPAEC-PAD elution profiles of nine cell wall monosaccharides in a concentration of 0.1µg/µl for each sugar.

1 = fucose, 2 = rhamnose, 3 = arabinose, 4 = galactose, 5 = Glucose, 6 = xylose & mannose, 7 = galacturonic acid, 8 = glucuronic acid.

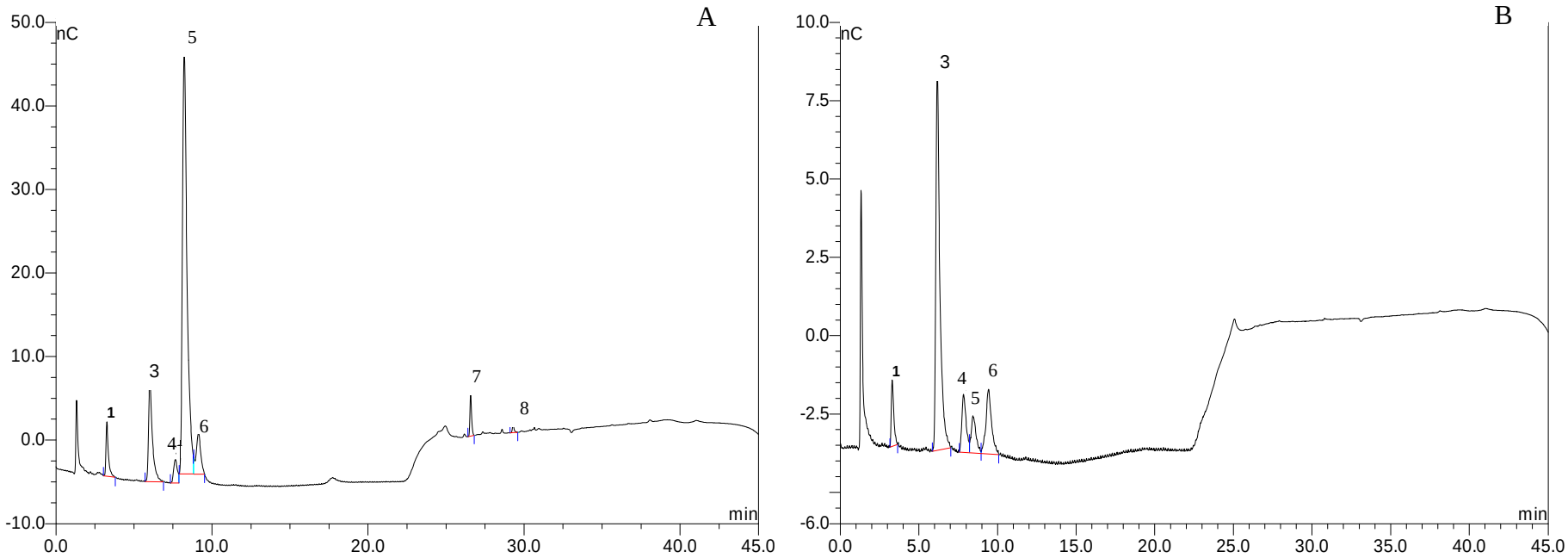

Figure 5.4. HPAEC-PAD elution profiles of representative WIP monosaccharides (A) and WSP monosaccharides (B) of eight types of canned beans.

1 = fucose; 3 = arabinose; 4 = galactose; 5 = Glucose; 6 = xylose & mannose; 7 , 8 = un-identified peak.

5.3.1 Effect of cultivar and region on the monosaccharides content of WIP and WSP.

Table 5.4 shows the amount of detected monosaccharides which was hydrolysed from WIP and WSP for the eight types of canned beans. The yield of hydrolysis was low ranging from 0.915 – 2.034g of total dietary fibre monosaccharides per 100g of canned beans (wet weight basis). For WIP, glucose represented around 60% of the WIP (0.485 – 1.407g / 100g bean, wet weight basis) and most of it was believed to be derived from non-digested starch including resistant starch, certain amount may be derived from hemicelluloses such as xyloglucan. This indicated that the reason for higher amount of dietary fibre determined in this project may be due to the starch not being fully hydrolysed. The next most abundant monosaccharide was arabinose, WIP contained 0.161 – 0.301g arabinose per 100g of beans (wet weight basis). Xylose & mannose and galactose present in lowest amount in WIP at 0.075 – 0.123g and 0.055 – 0.114g per 100g of beans (wet weight basis) respectively. For WSP, arabinose was most abundant at 0.050 – 0.094g per 100g of beans (wet weight basis). The next most abundant component was xylose & mannose (0.007 – 0.016g/100g bean, wet weight basis) and followed by galactose (0.009 – 0.021g/100g bean, wet weight basis). Very small amount of glucose in WSP may come from non-digested starch or xyloglucan.

The reason for low or no uronic acid was detected in dietary fibre could be two possibilities: first, 2M TFA at 120°C for 1h gives efficient release of neutral, non-cellulosic sugar and little decomposition of free monosaccharides (Fry, 1988), so that it did not completely hydrolyse the uronic acid linkages. Second, the main resource for uronic acid is from the backbones of HG, RGI and RGII which are linked together. The backbones have a strong structure especially the un-methylesterified HG is cross-linked with calcium which makes the structure even stronger. However, TFA is weak acid, it is only efficient for neutral sugar to hydrolyse bonds on the side chains of RGI and capture the free arabinan and galactan. TFA is not strong enough to hydrolyse the backbone for HG, RGI and RGII. So that uronic acid was still existing as polysaccharides which was solubilised but not hydrolysed to monosaccharides. Therefore, other methods need to be used to analyse the uronic acid content. It was suggested to hydrolyse for 4 - 24h at 100°C

in 70 - 90% formic acid in which uronic acids are relatively stable (Fry, 1988), or use H_2S0_4 at 70°C to hydrolyse the dietary fibre (Scott, 1979).

Table 5.4. Amount of detected monosaccharides (g) in 100g of canned beans (wet weight basis) from eight types of canned beans.

		Cell wall monosaccharides (CWM)					
		Arabinose	Galactose	Xylose & Mannose	Total CWM [A]	Glucose [B]	Total [C]
A1	WIP	0.161[a]	0.055	0.075	0.418	0.485	0.915
	WSP	0.094	0.016	0.017		0.012[a]	
B1	WIP	0.211[a]	0.073	0.090	0.482	0.677	1.167
	WSP	0.067	0.020	0.021		0.008[a]	
C1	WIP	0.245[a]	0.100	0.123	0.571	0.966	1.549
	WSP	0.072	0.015	0.016		0.012[a]	
D1	WIP	0.183[a]	0.077	0.093	0.432	0.599	1.038
	WSP	0.058	0.010	0.011		0.007[a]	
A2	WIP	0.256[b]	0.097	0.109	0.516	0.710	1.231
	WSP	0.039	0.007	0.008		0.005[b]	
B2	WIP	0.252[b]	0.084	0.100	0.504	0.633	1.143

	WSP	0.050	0.009	0.009	0.006[b]	
C2	WIP	0.301[b]	0.114	0.114	1.407	2.034
				0.618		
	WSP	0.064	0.012	0.013	0.009[b]	
D2	WIP	0.214[b]	0.082	0.111	0.437	0.959
				0.514		
	WSP	0.079	0.013	0.015	0.008[b]	

Values show mean of 3 replicates. Letters a, b means within same column letters are not significantly different at $p<0.05$ levels.

A. Total CWM (cell wall monosaccharides)= arabinose + galactose + xylose & mannose
B. Glucose was derived from non-digested starch. Small amount was derived from xyloglucan.
C. Total detected monosaccharides from hydrolysed dietary fibre = cell wall polysaccharides + glucose

In order to investigate the cultivar and region effect on the monosaccharides in WIP and WSP, two - factor ANOVA was carried out (Table 5.5). Although numerically C2 had the largest total amount of cell wall monosaccharides and total amount of detected monosaccharides, A1 had the least, but no statistic significant were found at 95% confidence level. However, when consider it as 90% confidence level, region were found to be significantly affect the total amount of cell wall monosaccharides and total amount of detected monosaccharides (Table 5.5). Beans from region C had significant higher content of total cell wall monosaccharides and total amount of detected monosaccharides than region A and D (Table 5.4).

For the total cell wall monosaccharides (arabinose + galactose + xylose & mannose), at 95% confidence level, cultivar was only found to have significant effect on the arabinose content of WIP (Table 5.5), cultivar 2 had higher amount of arabinose than cultivar 1 (Table 5.4). At 90% confidence level, cultivar was found to have significant effect on the galactose content of both WIP and WSP (Table 5.5),

cultivar 2 had higher amount of galactose in WIP and lower amount of galactose in WSP than cultivar 1 (Table 5.4). Region did not have effect on any cell wall monosaccharides at 95% confidence level, but significantly affect galactose content in WIP at 90% confidence level (Table 5.5). For glucose, cultivar was found to significantly affect the glucose content in WSP. Cultivar 2 had less glucose in WSP than cultivar 1.

Table 5.5. Two - factor ANOVA P-value for the effect of cultivar and region on the monosaccharides of WIP and WSP.

		Cultivar	Region	Cultivar : Region
Arabinose	WIP	0.02	0.1	0.66
	WSP	0.11	0.75	0.07
Galactose	WIP	0.06	0.054	0.13
	WSP	0.054	0.70	0.16
Glucose	WIP	0.23	0.07	0.59
	WSP	0.04	0.053	0.64
Xylose & Mannose	WIP	0.13	0.11	0.13
	WSP	0.84	0.83	0.17
Total CWM [A]		0.10	0.07	0.85
Total [B]		0.20	0.07	0.75

At 95% confidence interval

A. Total CWM = arabinose + galactose + xylose & mannose
B. Total detected monosaccharides from hydrolysed dietary fibre = cell wall polysaccharides + glucose

Figure 5.5 shows the correlation between total monosaccharides and glucose content in dietary fibre of canned beans. There is a strong linear positive relationship between them (r=0.83). So that the more cell wall monosaccharides the beans had, the more glucose content in the dietary fibre. Because most of the glucose content in dietary derived from non-digested starch. It indicated that the more cell wall monosaccharides the beans had, the more difficult for the enzyme to digest the starch.

No reports have studied the cultivar or region effect on the composition of dietary fibre in canned baked beans. But Srisuma et al. (1991) has studied the cultivar effect on the cell wall monosaccharides for the WSP of raw beans, they found that Fleetwood had less xylose and galactose compared to the other cultivars, and suggested beans from Fleetwood had more side chains with a shorter average chain length. Canned beans dietary fibre constituent is not comparable to raw beans because the thermal treatment may change the polysaccharides structure by degradation (Stolle-Smits et al., 1995).

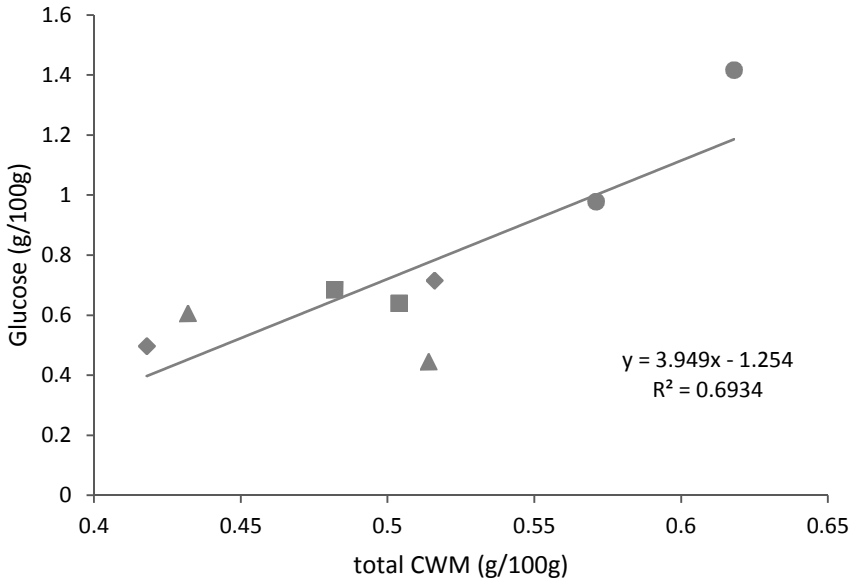

Figure 5.5 correlation between total cell wall monosaccharides content and glucose content from dietary fibre of canned beans.

5.3.2 The cell wall monosaccharides content in WIP and WSP.

In order to investigate the cell wall monosaccharides constituent in WIP and WSP, the monosaccharides compositions were expressed as the weight percentage of the detected amount (Table 5.6). Because the glucose was derived from non-digested starch, the data was corrected in the calculation excluding the glucose. Excluded glucose, arabinose presented predominantly in both WIP and WSP (52.3% - 56.6% and 62.3% - 73.6%, w/w % respectively). The xylose & mannose present secondly dominant (22.0% - 27.2% and 13.7% – 19.2%, w/w % respectively) and followed by galactose (19.5% - 22.2% and 12.3% - 13.9%, w/w % respectively). Both xylose & mannose and galactose represented higher percentage in WIP than in WSP, this may contributed to the hemicellulose in WIP. Hemicellulose largely presented in cotyledon dietary fibre (17% - 22%, w/w %) (Srisuma et al., 1991) which included polysaccharides such as xylan, glucomannan, mannan, galactomannan or xyloglucan. Thus it is believed that the higher percentage of xylose & mannose and galactose in WIP was from monosaccharides which hydrolysed from hemicellulose.

Table 5.7 shows comparison of the cell wall monosaccharides percentage distribution (w/w %) of WIP and WSP of navy beans from different literatures. Shiga et al.(2006) claimed that for the WIP of the cotyledon, arabinose is the most abundant monosaccharide (56%), followed by xylose & mannose (13.5%), galactose (6%). For the WSP of cotyledon, arabinose is the most abundant monosaccharide (42%), followed by xylose & mannose (24%) and galactose (11%). Srisuma et al. (1991) studied the monosaccharides content of WSP from bean cotyledon, and claimed that arabinose was the predominant neutral sugar (64%), followed by xylose & mannose (15.5%) and galactose (11%). Therefore, regardless the processing effect, arabinose was most abundant in both WIP and WSP, followed by xylose & mannose, galactose.

Table 5.6. Cell wall monosaccharides percentage distribution (w/w %) of WIP and WSP from eight types of canned beans. [A]

		Arabinose	Galactose	Xylose & Mannose
A1	WIP	54.7	19.5	25.8
	WSP	73.5	12.8	13.7
B1	WIP	55.8	20.1	24.1
	WSP	62.3	18.5	19.2
C1	WIP	52.1	21.7	26.2
	WSP	70.2	14.3	15.5
D1	WIP	51.3	22.2	26.5
	WSP	73.6	12.4	14.0
A2	WIP	54.9	21.3	23.8
	WSP	71.3	13.8	14.9
B2	WIP	56.6	20.2	23.2
	WSP	73.1	13.0	13.9
C2	WIP	56.3	21.7	22.0

	WSP	71.0	13.9	15.1
D2	WIP	52.3	20.5	27.2
	WSP	73.4	12.3	14.3

A. Glucose has been excluded.

Table 5.7. Comparison of the cell wall monosaccharides percentage distribution (w/w %) of WIP and WSP of navy beans from different literature[A]

		This project	(Shiga and Lajolo, 2006)	(Srisuma et al., 1991)
		Canned bean	Raw bean	
WIP	arabinose	55	56	N/A
	Xylose & mannose	24	13.5	N/A
	galactose	21	6	N/A
WSP	arabinose	68	42	64
	Xylose & mannose	19	24	15.5
	galactose	13	11	11

A. Data was the average value adopted from different reports.

5.3.3 Hydrolysis of the dietary fibre (WIP + WSP) and the detection of the monosaccharides

The dietary fibre is not completely hydrolysed by 2M TFA. The hydrolysis percentage for dietary fibre is shown in Table 5.8, 2M TFA hydrolysed 22% - 47% (w/w %) of the WIP and 77% - 92% (w/w %) of the WSP. For WIP, the low hydrolysis percentage may due to the high amount of cellulose, ash, lignin that did not hydrolysed in TFA, which is thought to contribute approximately 48% (w/w %) of the WIP (Srisuma et al., 1991). The un-hydrolysed polymer for WIP is also likely to contain uronic acid that did not completely hydrolysed in 2M TFA (Fry, 1988). For WSP, it was hydrolysed to a larger extent than WIP, because WSP did not contain cellulose which cannot be hydrolysed.

The detected percentage was calculated as the ratio of the amount of detected monosaccharides to the amount of WIP / WSP which had been hydrolysed. WIP had a detected percentage of 19.5% - 36.9% (w/w %) and WSP only reached 7.5% - 11.1% (w/w %).

As discussed above, 2M TFA only efficient for neutral sugar to hydrolyse bonds on the side chains of RGI and capture the free arabinan and galactan. It is not strong enough to hydrolyse the backbone, where the uronic acid was derived from, to monosaccharides. Thus, the uronic acid was probably solubilised in the solution as polysaccharides, but not detectable by the HPAEC-PAD.

WIP contained 32% of hemicellulose (Srisuma et al., 1991). So it is believed that the hydrolysed WIP contained approximately 30% hemicellulose and 70% pectic polysaccharides. It is known that 70% of the pectic polysaccharides were composed of uronic acid (Mohnen, 2008). Hence, uronic acid contributed approximately 50% of WIP. Therefore, the majority of undetected constituent in WIP was believed to be uronic acid (50%, w/w %) which was not hydrolysed to monosaccharides but solubilised as polysaccharides. The other undetected constituent may be rhamnose that exist in the backbone of RGI and some protein in the cell wall.

WSP was made of pectic polysaccharides and some of the hemicellulose. Because of 70% of the pectic polysaccharides were composed of uronic acid (Mohnen, 2008), so that around 70% of the undetected constituent in WSP may be uronic acid which was not hydrolysed but solubilised. Same as WIP, the other undetected constituent

may be rhamnose that exist in the backbone of RGI and some protein in the cell wall.

Besides, the detected percentage were also affected by the limit of detection (LOD) and limit of qualification (LOQ). LOD and LOQ for different sugars were shown in Table 2.2. Some of the composition maybe not detected because its level was below LOD. According to Srisuma et al. (1991), raw navy bean galactose ratio to rhamnose is 4 in soluble polymer. So that we inferred that in navy bean, rhamnose content is around 1.8×10^{-4}μg/μl. The LOD for rhamnose is 3.91×10^{-4}μg/μl, hence, the rhamnose cannot be detected due to the small amount. Hence small amount of undetected composition may be the tiny amount of rhamnose that was hydrolysed by TFA.

Above all, the un-hydrolysed dietary fibre was composed of predominant constituent – cellulose and certain amount of un-hydrolysed uronic acid, lignin, cell wall protein and ash. The un-detected monosaccharides in test sample were composed of predominant constituent – uronic acid, certain amount of cell wall protein and small amount of rhamnose.

Table 5.8. Hydrolysis percentage for dietary fibre (w/w %) and detected percentage for test sample (w/w %).

%		A1	B1	C1	D1	A2	B2	C2	D2
Hydrolysis percentage[A]	WIP	25.4	24.2	37.1	44.8	41.9	22.0	47.4	38.3
	WSP	81.2	91.8	77.2	77.2	80.1	87.6	83.4	78.9
Detected percentage[B]	WIP	33.1	36.9	28.6	19.5	22.9	35.2	27.5	20.5
	WSP	11.1	7.8	10.2	10.7	9.9	8.0	7.5	10.3

A. Percentage of hydrolysed dietary fibre out of the total.

B. Percentage of detected monosaccharides out of the dietary fibre that was hydrolysed.

5.4 Effect of cultivar and region on the starch content of canned beans

Starch is the most abundant carbohydrate in the legume seeds such as black grams, chick pea, lentils, and kidney bean. They contain 38% - 48% starch out of 50% - 60% carbohydrate (Rehman and Shah, 2005, Berg et al., 2012). In this section, the starch content of canned navy beans will be discussed, and investigate the cultivar and region effect on the starch content of canned beans.

The starch of canned beans was digested by enzymes α-amylase (Termamyl) and amyloglucosidase. The supernatant was determined using two methods: HPAEC-PAD and 3, 5-Dinitrosalicylic acid (DNS) method. HPAEC-PAD detected the glucose hydrolysed from starch and DNS detected the reducing sugar including glucose and probably maltose and soluble maltodextrins. Figure 5.6 shows the standard HPAEC-PAD elution profile of glucose in a concentration of 0.005µg/µl with internal standard fucose. The retention time for glucose is 4.17min, with an area of 6 nC*min. The starch content can be calculated by glucose content, as glucose × 0.9 (Cho, 1999). The factor 0.9 converts grams of glucose to grams of the starch, is calculated by dividing the molecular weight of glucose minus one molecule of water (180-18) by the molecular weight of glucose (180) (University of Wisconsin, 2007).

The amount of starch content for eight types of canned beans was shown in Figure 5.7. Canned beans contained 9.14 – 11.48g starch content in every 100g of beans (wet weight basis). Canned beans contained 63% of water. So when calculated as dry weight basis, canned beans contained 23.5% - 31.7% (dry weight basis) starch content in 100g of beans. As discussed above, dietary fibre contained approximately 0.5 – 1.4g non-digested starch per 100g of beans. Hence, when calculated the non-digested starch into consideration, canned beans contained 10.2 – 11.9g starch in every 100g of beans (wet weight basis), 27.5 – 32.13g starch in every 100g of beans (dry weight basis). Numerically C2 had highest amount of starch and D2 had the lowest amount.

Navy bean was found to have a lower proportion of starch compare to other legumes due to its higher fine fibre content (Hoover and Ratnayake, 2002). No studies were

found to determine the starch content in canned baked beans. Table 5.3 shows some reports studied the starch content in navy beans. Costa et al. (2006) claimed beans soaked and boiled in water contained 33.5% (w/w %, dry weight basis) of starch content. Few reports had studied on the starch content for raw navy beans. Raw navy beans contain around 29.2% (w/w %, dry weight basis) of starch out of 56% (w/w %, dry weight basis calculated by difference) of carbohydrate (Costa et al., 2006). A range of 18% - 36% (w/w %, dry weight basis) of starch for raw beans were determined by different projects (Kereliuk and Kozub, 1995, Hoover and Ratnayake, 2002, Lowe, 2012). The results observed in this project 24.68% - 31.0% (dry weight basis) is within the range of other projects. As discussed in section 5.2, different results could be due to different methods applied, different samples used, also the different processing would significant influence the results.

The DNS method detected less reducing sugar compared to HPAEC-PAD, 3.71g – 4.56g reducing sugar in every 100g beans (wet weight basis) was detected including glucose and maltose (Figure 5.8). However, it was much less than the glucose amount detected by HPAEC-PAD (10.15g – 12.75g/100g bean, wet weight basis, data not shown). Hence, it is believed that HPAEC-PAD method is more sensitive to determine the starch content. Therefore HPAEC-PAD is recommended to use to determine the monosaccharide in starch hydrolysed material.

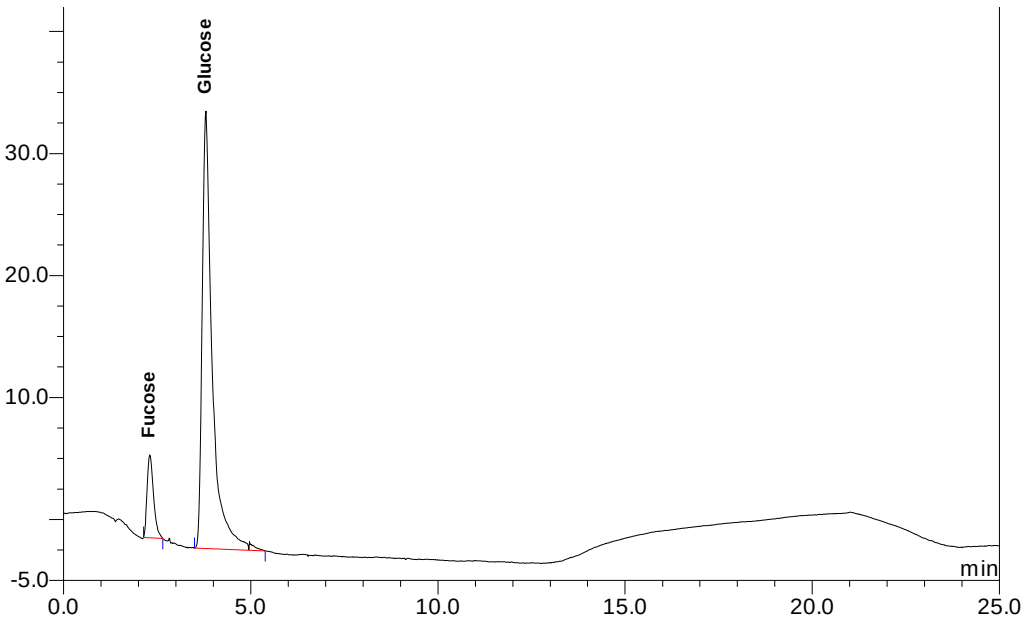

Figure 5.6. Standard HPAEC-PAD elution profiles of glucose in a concentration of 0.005µg/µl with internal standard fucose.

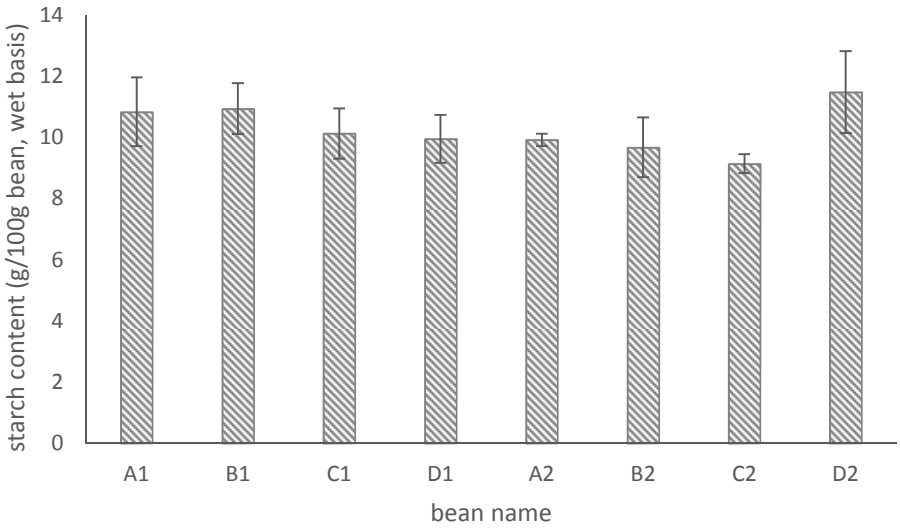

Figure 5.7 Starch content (g/ 100g. wet weight basis) in the canned beans of eight types of beans.

Values show mean of 3 replicates +/- standard error of mean at 95%confidence interval.

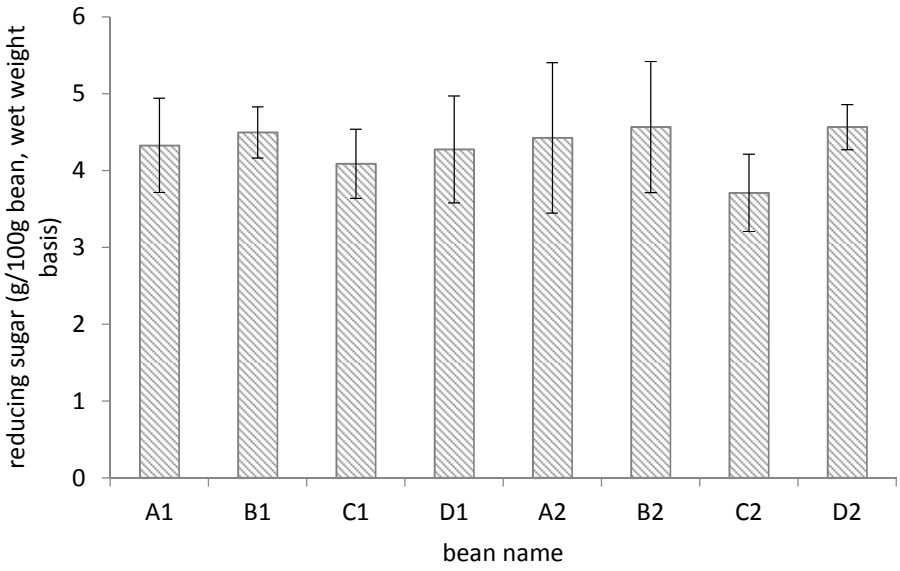

Figure 5.8. Reducing sugar content (g/ 100g. wet weight basis) in the canned beans of eight types of beans detecting by DNS.

Values show mean of 3 replicates +/- standard error of mean at 95% confidence interval.

In order to investigate the effect of cultivar and region effect on the amount of starch content, a two factor ANOVA was carried out (Table 5.9). Although starch content among difference cultivars and regions is not statistic significant at 95% confidence interval because of variance, but region had significant effect at 90% confidence interval, beans from A and D had higher amount of starch than B and C. No significant difference was found between different cultivar and region.

Although there were no reports studied the cultivar or region effect on canned baked beans, but raw beans have been reported. Kereliuk et al. (1995) claimed that starch content was significantly influenced by region (Ontario had higher amount of starch than Alberta). Hoover et al. (2002) did not find differences among cultivars on the starch content of raw navy beans, but found cultivar had significant effect on black beans and peas starch content. Therefore, the effect of cultivar and region on starch content of legumes was species and cultivar dependent. Canning process also may have significant effect on the starch content.

Table 5.9. Two - factor ANOVA P-value for amount of glucose in canned beans of eight types of beans using HPAEC-PAD and DNS methods.

	HPAEC-PAD	DNS
Cultivar	0.55	0.80
Region	0.72	0.063
Cultivar : Region	0.45	0.10

At 95% confidence interval

5.5 Conclusion and discussion

Canned baked beans were rich in dietary fibre (10.8% - 16.4% (w/w %, wet weight basis)), especially WIP (9.2% - 14.8% (w/w %, wet weight basis)). Beans from B and C (average 14.9%, w/w %, wet weight basis) had significant higher amount of

dietary fibre than A and D (average 11.9%, w/w %, wet weight basis). Cultivar 2 (average 13.9%, w/w %, wet weight basis) had higher fibre content than cultivar 1 (average 13%, w/w %, wet weight basis). Cultivar and region had significant effect on WIP content with interaction, C2 (14.8%, w/w %, wet weight basis) had higher WIP than A1 (9.2%, w/w %, wet weight basis). The effect of cultivar and region on the dietary fibre content of canned baked beans has been investigated. Although Kereliuk and Kozub (1995) found that region had significant effect on the fibre content of raw beans; beans from Ontario (similar to C) had lower amount of fibre than Alberta. Firmest beans (C2, 16.4%, w/w %, wet weight basis) had significant higher amount of dietary fibre than softest beans (A1, 10.8%, w/w %, wet weight basis). It appears that firmer beans have lower proportion of WSP. This supports the previous observations and the literature that soluble pectin may have a role in determining bean firmness. A1 had a ratio of WSP:WIP 0.44, C2 had a ratio of WSP:WIP 0.17, therefore, firm beans had low pectin solubility than soft beans.

Dietary fibre was composed of cell wall polysaccharides and resistant starch. The fibre was hydrolysed to investigate the cell wall monosaccharide composition of the fibre. Arabinose presented predominantly in dietary fibre (approximately 60%), xylose & mannose present secondly dominant (approximately 20%), followed by galactose (approximately 17%), the rest including small amount of glucose. C2 which was considered as a firm bean was found to contain more cell wall monosaccharides, A1 which was considered as soft beans was found to contain less cell wall monosaccharides. Region had significant effect on hydrolysed cell wall neutral sugar content at 90% confidence interval. Region C had higher amount of neutral sugar than region A. Cultivar was found to have significant effect on arabinose content in WIP at 95% confidence interval, and on galactose in both WIP and WSP at 90% confidence interval. Cultivar 2 had more arabinose and galactose in WIP but less galactose in WSP. Region was found to have effect on arabinose and galactose in WIP at 90% confidence interval. Beans from region C had more neutral sugar, and were suggested to have longer side chain length (Figure 5. 9. B) or more branched side chains (Figure 5. 9. A), and these longer or more side chains may contribute to the firmness of the beans because the pectin may be more difficult to break down or solubilize. In contrast, beans from region A which had least neutral sugar had the softest texture. As suggested by Srisuma et al. (1991), neutral sugar

content is related to the side chain length in RGI. They found cultivar Fleetwood had significantly less neutral sugar compared to other cultivars, and in the precipitation steps during cell wall material isolation (WSP), Fleetwood showed gel-like transparent characteristics rather than precipitation for other cultivars. Therefore, higher amount of neutral sugar in the firmer beans may have longer side chain (Figure 5. 9 B) or more branched side chains (Figure 5. 9 A).

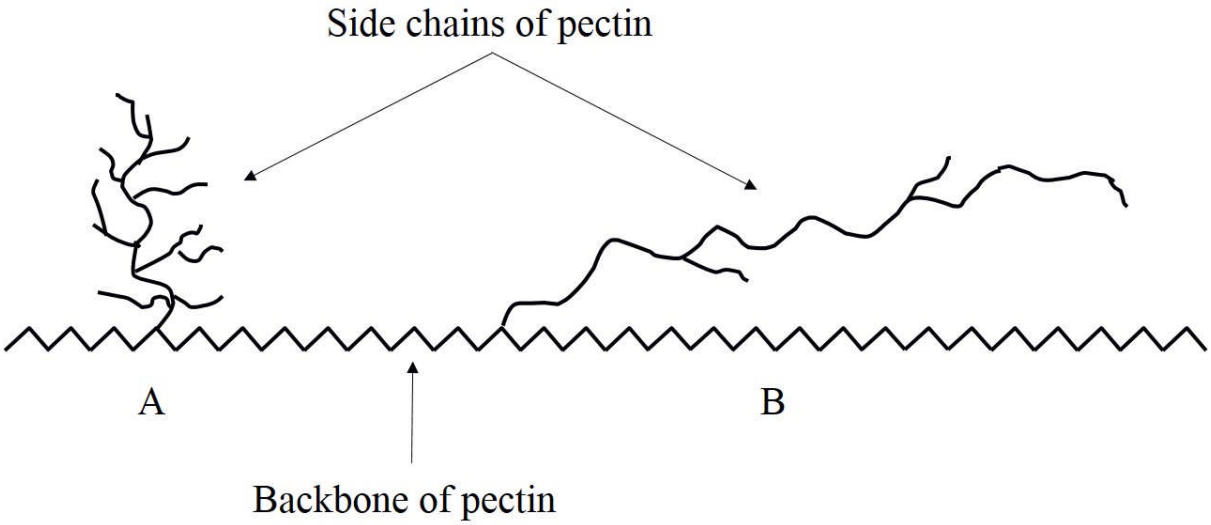

Figure 5. 9 Diagram of the structure of pectin with side chains. A and B are two different types of side chains of pectin.

The firmness of beans was found highly correlated with total neutral sugar (r=0.8**) in WIP especially arabinose (r=0.8**) and galactose (r=0.75**). This result was comparable with the results found by Shiga et al. (2004). The hardening of the beans was claimed to involve insoluble neutral, Ara-rich polysaccharides (Shiga et al., 2004). Shiga et al. (2004) suggested that Ara and Gal residues in pectin were usually found esterified by ferulic acid, and this ether bond formation between a phenolic –OH group and a –OH group of polysaccharides could be involved in the wall polymer cross linking and insolubilization (Shiga et al., 2004), therefore involved in the hardening of the beans. The interactions between Ara-rich polymers were associated with storage conditions (temperature and humidity), therefore, it is possible that the climate in different regions (temperature and humidity) would have significant effect on the insolubilisation of the Ara/Gal-rich polymers found in WIP, and therefore significantly affect the firmness of the beans. However, this observation on extracted and hydrolysed polymers is not supported by the semi-

quantitative analysis of soluble polymers using ELISA (which would discuss in chapter 6).

Lower amount of starch were detected (9.14% - 11.48% (w/w %, wet weight basis)) compared to other reports, and it may due to the abundant presence of dietary fibre. It is believed that starch from legumes is difficult to isolate due to the presence of fibre derived from cell wall enclosing the starch granules (Hoover and Ratnayake, 2002, Hoover and Sosulski, 1985), beans with higher amount of fibre content (C2) contained lower amount of starch compared to other beans. Beans from B and C had lower amount of starch than A and D at 90% confidence interval.

A High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) method was developed to determine the monosaccharides in the beans. In this study, it was found to be more sensitive in detecting carbohydrate, because according to the results, HPAEC-PAD detected 2.7 times more glucose than DNS method. Cell wall sugars are very hard to separate, and the hydrolysis conditions significant affect the detection. 2M TFA was only efficient at hydrolysing a small portion of the neutral sugar in the cell walls but not the other cell wall polysaccharides. Stronger acid is needed for the hydrolysis to determine the other cell wall polysaccharides, such as cellulose and uronic acid containing pectin. HPAEC-PAD worked very well at determining the oligosaccharides such as raffinose stachyose and verbascose. The industry can measure the free sugar and glucose using HPAE-PAD with very easy access. An advantage of HPAEC-PAD is that is not affected by polyphenols, which inhibit a few of the other carbohydrate assays available.

This chapter discussed the carbohydrate composition in the canned beans. Since beans canning in different medium were found to have different mechanical properties, it is believed that exchange happened between beans and medium, soluble component leach from beans into sauce, as well as soaked in soluble component from sauce into beans. Therefore, in the next chapter, the soluble component exchange during processing will be discussed.

Chapter 6
Exchange of soluble pectin and oligosaccharides between beans and medium during processing

6.1 Introduction

Food cooking and canning in syrup was found not only exhibit an uptake of carbohydrate by diffusion but also appreciable adsorption on the cell walls take place (Kramer and and Szczesniak, 1973). The results presented in chapter 3 showed that different cooking media significantly affected the firmness of beans. Beans canned in tomato sauce were 34% - 39% firmer than beans canned in water or brine. The viscosity of the canning medium was inversely proportional to bean hardness. The results presented in chapter 4 showed that different canning media had effects on the intercellular matrix which would then affect the cell separation. Thus, these results indicated that interactions between beans and sauce happened during processing and these interactions significantly affected the beans and sauce properties. Therefore, this chapter will investigate the solubility of pectin into the canning medium during processing. Figure 6.1 shows the flow chart of the extraction of soluble pectin and oligosaccharides.

In this chapter, the aims were: 1) to evaluate the soluble pectin from beans leaching into surrounding medium during blanching and canning; 2) to evaluate the solubility of oligosaccharides (raffinose and stachyose) during canning; 3) to investigate the effect of growth region and cultivar on the soluble pectin and oligosaccharides (raffinose and stachyose).

Beans were blanched in tap water and thereafter canned in tomato sauce, water or brine. The processing liquids were collected to measure the yield of soluble pectin and oligosaccharides (raffinose and stachyose). ELISA was a method using immune-probe (antibody JIM5, JIM7, LM5 and LM6) to detect the soluble pectin (un-methylesterified HG, methylesterified HG, galactan and arabinan) in the beans which leached into the medium. HPAEC-PAD was used to measure the oligosaccharides (raffinose and stachyose) in the sauce in order to calculate the exchange of oligosaccharides during canning.

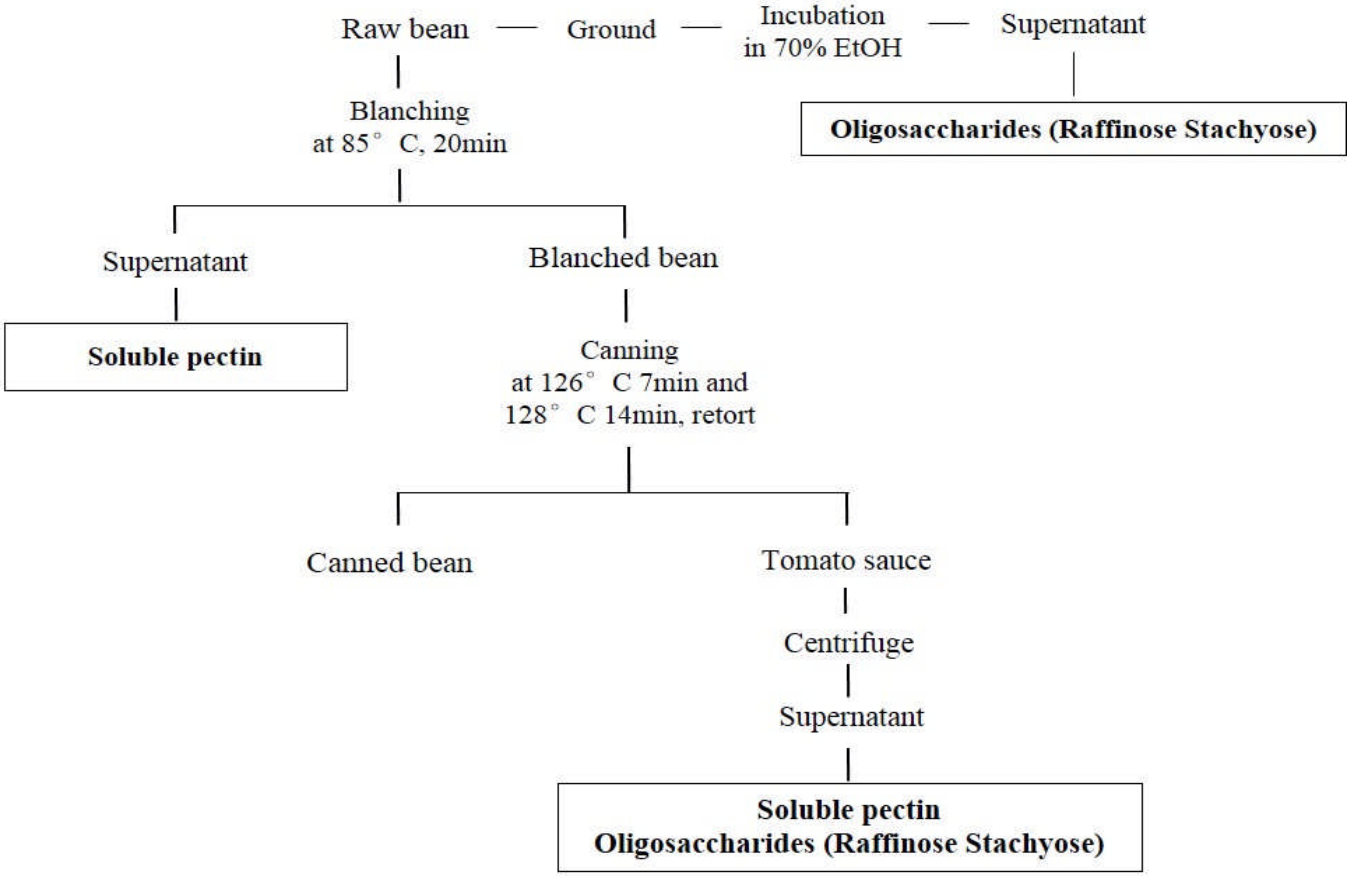

Figure 6.1. General flow chart of extraction of oligosaccharides (raffinose and stachyose) for raw bean, soluble pectin for blanched bean, soluble pectin and oligosaccharides (raffinose and stachyose) for canned bean.

6.2 Solubility of pectin epitopes during blanching and canning

Beans were processed under blanching and canning. During the processing, the exchange between the soluble component in the beans and in the sauce happened. Soluble pectin were determined using pectin epitopes probes. ELISA was used for the detection of soluble pectin. Figure 6.2 shows the flow chart of the procedure for determining the soluble pectin in the beans. For blanched beans, the supernatant of the blanched water was determined. For canned beans, canned sauce were centrifuged and the supernatant was determined. The supernatant was bind on the surface of the microplate, then primary antibody which would capture the pectin in the supernatant was applied, the secondary antibody which attached with enzyme would recognise the primary antibody, and the colour changed from transparent to blue. After the colour development was stopped by the substrate H_2SO_4, colour changed from blue to yellow, then the absorbance were determined at 450 nm in a microplate reader. Four probes were used to detect the soluble pectin including un-methylesterified HG (JIM5 epitopes), methylesterified HG (JIM7 epitopes), galactan (LM5 epitopes) and arabinan (LM6 epitopes). This method is semi-quantitative, because it only provided the relative amount of the soluble pectin (the absorbance reading) to compare the differences amongst different beans, but it was not the accurate quantitative amount of soluble polysaccharides in beans with unit of gram. So it is not comparable between different polysaccharides because the microplate surface may have different preference binding for different antigens (pectins).

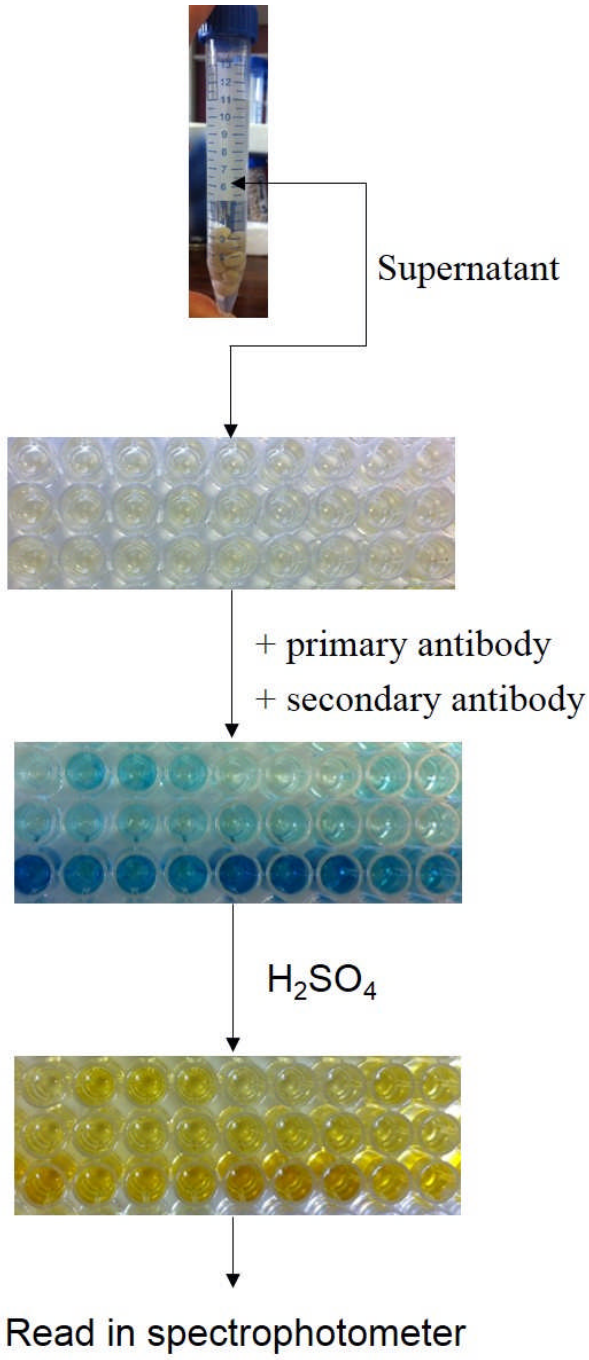

Figure 6.2. Flow chart of determination of soluble pectin of beans using ELISA.

Table 6.1 shows the absorbance reading of the soluble pectin leaching from the beans into the water during blanching, and the pectin in the sauce after canning. Compared to original sauce, un-methyl esterified HG increased in a very small amount (15%) which indicated that most of the soluble un-methyl esterified HG was lost during blanching. The transformation between un-methyl esterified and methyl esterified HG can be excluded because the high temperature blanching would deactivate the enzyme pectin methylesterase (PME) which demethylates cell wall pectins (Anthon and Barrett, 2006). Methylesterified HG decreased 3% - 43% compared to original sauce except C2. It has been studied that ripen tomato fruits contained high level of methyl esterified HG because of the PME reduced the level of bound calcium (Tieman et al., 1992, Orfila et al., 2001). It indicated that water soluble methylesterified HG lost from beans into water during blanching and in the canning process part of the water soluble methylesterified HG in the sauce diffused into the beans. A1 which is the softest bean soaked in highest amount of methyl esterified HG from the sauce.

Galactan decreased up to 64% compared to original sauce. It indicated that most of the water soluble galactan lost from beans into water during blanching. During canning process part of galactan in the sauce diffused from sauce into beans. This may explain why the beans canned in tomato sauce were firmer than canning in the water or brine (obtained from Chapter 3). It is found that RGI side chains include galactan would contribute to cell adhesion (Caffall and Mohnen, 2009), and pea cotyledon which contained high level of galactan in the late in development in the cell wall was found to lead to a firmer texture (McCartney et al., 2000). Thus, it is possible that the galactan which were soaked into the beans from the sauce made the beans texture firmer.

Arabinan increased up to 53% compared to original sauce. Arabinan is a very flexible, and water soluble polysaccharides (Renard and Jarvis, 1999). So the increasing indicated that although a quite amount of arabinan already lost from beans into water during blanching, it still have a large amount of water soluble arabinan lost during canning. All the diffusions for the pectins were believed to be affected by the osmotic pressure from the tomato sauce.

For blanched beans, A1 and C2 had significant lower amount of un-methyl esterified HG (labelled with JIM5). The other beans had up to 37% higher amount of un-methyl esterified HG than A1 and C2. B2 had significant higher amount of methyl esterified HG (labelled with JIM7) and A1 and C2 had significant lower amount compare to the other beans. B2 which had highest amount of methyl esterified HG had up to 73% of methyl esterified HG more than the A1 and C2 which had the lowest amount. A1 had significant highest amount of galactan (labelled with LM5) compare to the other beans. C2 had significant highest amount of arabinan (labelled with LM6) followed by A1, B2 and D2. In summary, A1 and C2 both had relative lower amount of HG but higher amount of galactan and arabinan; B2 had higher amount of HG and arabinan but lower amount of galactan.

For canned beans, D1 had significant higher amount of un-methyl esterified HG (labelled with JIM5) than C1, B2 and D2. C2 and A2 had higher amount of methyl esterified HG (labelled with JIM7) than other beans, the galactan of canned beans (labelled with LM5) did not significantly different among different beans, the arabinan (labelled with LM6) in cultivar 1 was significant lower than cultivar 2. But compare to the other beans, numerically A1 and C2 still got relatively higher amount of galactan and arabinan. Therefore, it showed that soluble pectin were not necessary correlated with the firmness of the beans because no obvious trends were found. The relationship between soluble pectin and mechanical properties will be discussed in chapter 7.

Table 6.1 Soluble polysaccharides leaching into the blanched liquid and canned sauce measured with ELISA method (absorbance at 450nm).

	Blanched				Canned			
	JIM5[A]	JIM7[B]	LM5[C]	LM6[D]	JIM5[A]	JIM7[B]	LM5[C]	LM6[D]
A1	0.58[a]	0.29[a]	0.27[c]	2.96[c]	0.38[ab]	1.17[a]	0.66	1.03[a]
B1	0.87[c]	0.73[b]	0.20[a]	1.61[a]	0.38[ab]	1.46[b]	0.54	1.05[a]
C1	0.90[c]	0.62[b]	0.20[a]	1.69[a]	0.35[a]	1.80[c]	0.55	0.83[a]
D1	0.90[c]	0.67[b]	0.20[a]	1.84[a]	0.41[b]	1.54[b]	0.53	0.96[a]
A2	0.90[c]	0.70[b]	0.20[a]	1.90[a]	0.39[ab]	1.98[cd]	0.59	1.03[b]
B2	0.92[c]	1.01[c]	0.23[b]	2.33[b]	0.33[a]	1.53[b]	0.57	1.20[b]
C2	0.64[b]	0.27[a]	0.25[b]	3.45[d]	0.39[ab]	2.19[d]	0.59	1.24[b]
D2	0.85[c]	0.58[b]	0.24[b]	2.16[b]	0.35[a]	1.97[c]	0.65	1.30[b]
Original sauce					0.32	2.03	1.46	0.57

Un-methyl-esterified HG binding with antibody JIM5, methyl-esterified HG binding with antibody JIM7, galactan binding with antibody LM5 and arabinan binding with antibody LM6. Values show mean of 3 replicates of absorbance measured at 450 nm.

6.3 Effect of cultivar and region on the exchange of soluble pectin between beans and cooking medium during blanching and canning

In order to investigate the cultivar and region effect on the exchange of soluble polysaccharides between beans and medium, two - factor ANOVA was carried out (Table 6.2). The results show, for blanched beans, region had significant effect on un-methyl esterified HG (labelled by JIM5), interaction happened for JIM5 because in region A and B, cultivar 2 is higher than cultivar 1, however in region C and D cultivar 2 is lower than cultivar 1 (Table 6.1). Both cultivar and region had significant effect on methyl esterified HG (labelled by JIM7), interaction happened because in region A & B and C & D, cultivar 1 and cultivar 2 behaved in an opposite way (Table 6.1). Cultivar and region both had significant effect on galactan (labelled by LM5) and arabinan (labelled by LM6), interaction happened for LM5 and LM6, because in region A cultivar 2 was lower than cultivar 1, but other regions behaved oppositely (Table 6.1). In conclusion, for blanched beans, cultivar had significant effect on all the soluble polysaccharides except un-methyl-esterified HG. Region had significant effect on all the soluble polysaccharides. Interactions happened for all the soluble polysaccharides. However, relationship between soluble pectin and cultivar or region was not found.

For canned beans, cultivar and region had no significantly effect on un-methyl esterified HG (labelled by JIM5), however interaction happened significantly because in region A and C, cultivar 2 was higher than cultivar 1, however in region B and D it was the opposite way (Table 6.1). Cultivar, region and interaction all had significant effect on methyl esterified HG (labelled by JIM7), interaction happened because all cultivar 2 higher than cultivar except B2. Cultivar and region had no significant effect on galactan (labelled by LM5). Only cultivar had significant effect on arabinan (labelled by LM6), cultivar 2 beans had higher amount of water soluble arabinan than cultivar 1 in canned beans. in conclusion, for canned beans, cultivar had significant effect on methyl-esterified HG and arabinan. Region only had significant effect on methyl-esterified HG. Interactions happened for un-methyl-esterified HG and methyl-esterified HG. However, same as blanched beans, relationship between soluble pectin and cultivar or region was not found.

No reports were found to discuss the blanched beans soluble polysaccharides. There was one report that reported that the cultivar had effect on the soluble pectin content of canned beans. Total soluble pectin content of the canned beans was found significantly affected by cultivars, beans from Fleetwood had the most soluble pectin content (Wang et al., 1988). And no significant differences of soluble pectin content were found between different regions (Arthur and Hatton) (Lu and Chang, 1996). But the composition of pectin was not reported. Therefore, it is believed that the region or cultivar effect on soluble pectin is very species and geographical depended, they all need to discuss specifically for each specific cultivar and region.

Table 6.2 Two - factor ANOVA P-value for amount of polysaccharides in the hydration liquid of eight types of blanched beans using ELISA method (binding with different antibodies).

	Blanched				Canned			
	JIM5	JIM7	LM5	LM6	JIM5	JIM7	LM5	LM6
Cultivar	0.2	0.013	0.001	<0.001	0.1	<0.001	0.43	<0.001
Region	<0.001	<0.001	0.015	<0.001	0.2	<0.001	0.69	0.66
Cultivar : Region	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.43	0.22

At 95% confidence interval

6.4 Evaluation of oligosaccharide (raffinose and stachyose) exchange during canning

6.4.1 Effect of cultivar and region on the oligosaccharides (raffinose and stachyose) content of raw beans

Raffinose and stachyose are oligosaccharides commonly found in legumes and often result in flatulence in humans (Steggerd, 1968, Hellendo, 1969, Tanaka et al., 1975). In this section, raffinose and stachyose will be studied for raw beans and canned beans. Raw beans were ground to powder and incubated in 70% ethanol to extract the sugars (Chapter 2, section 2.7.3), supernatant was analysed using HPAEC-PAD. Canned sauce was centrifuged and supernatant was used to analyse the sugars by HPAEC-PAD.

Figure 6.3 shows the standard elution profile for raffinose and stachyose in HPAEC-PAD. Raffinose and stachyose elution time are 11.98min and 13.65min respectively. Figure 6.4 shows the representative HPAEC-PAD elution profiles of monosaccharides, disaccharides and oligosaccharide of raw beans. 7 peaks have been identified which are fucose (internal standard, peak 1), glucose (peak 2), fructose (peak 3), sucrose (peak 5), raffinose (peak 6), stachyose (peak 7) and verbascose (peak 8). Peak 4 is un-identified peak because no standard for it was done. However, according to Giannoccaro et al. (2008), it is possible that peak 4 is melibiose. Stachyose and sucrose presented the largest portion in raw beans oligosaccharides.

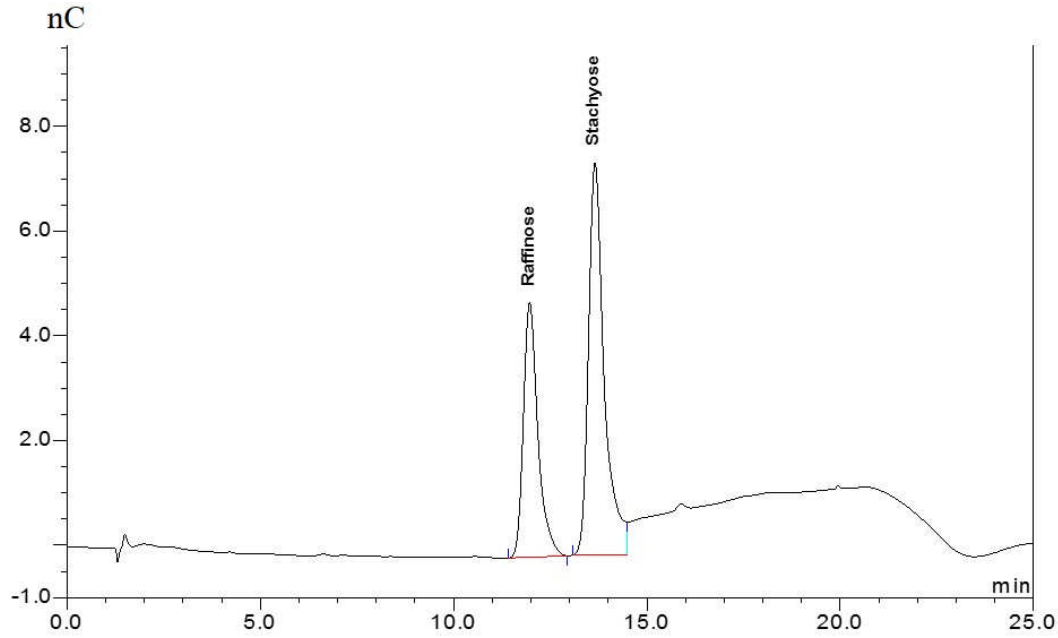

Figure 6.3. Standard HPAEC-PAD elution profiles of raffinose and stachyose at a concentration of 0.005µg/µl.

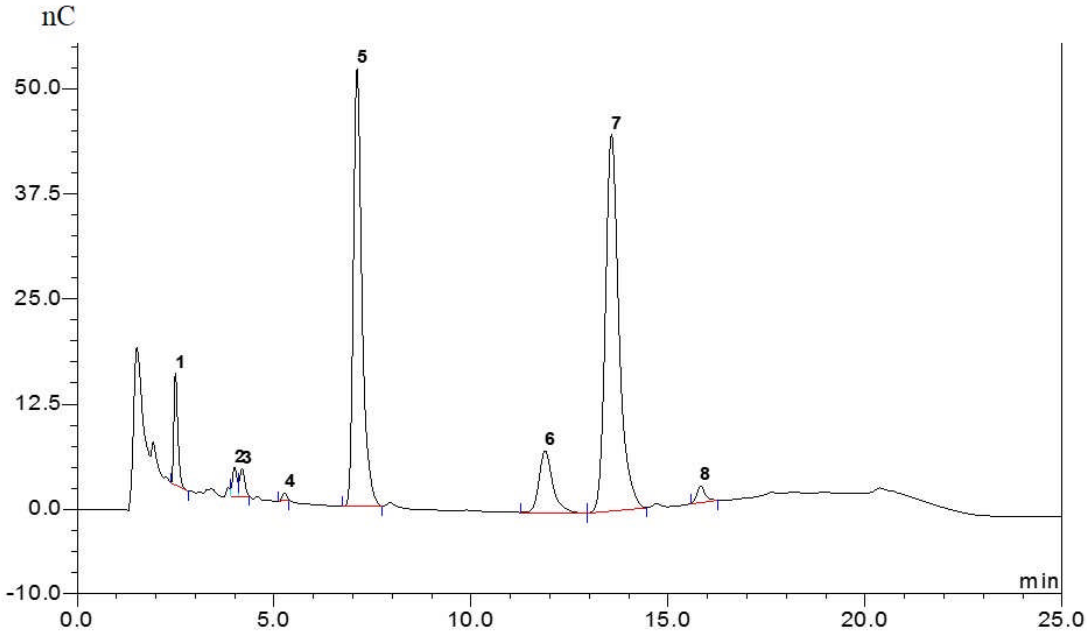

Figure 6.4 Representative HPAEC-PAD elution profiles of monosaccharides, disaccharides and oligosaccharide extracted from raw beans.

1 = fucose, 2 = glucose, 3 = fructose, 4= unidentified peak, 5 = sucrose, 6 = raffinose, 7 = stachyose, 8 = verbascose.

Seventeen types of raw beans oligosaccharides were extracted, and contained between 0.30 – 0.47g of raffinose and 1.65 – 2.48g of stachyose in every 100g of beans (dry weight basis) (Table 6.3). For eight types of beans, raw beans contained 0.38 – 0.47g of raffinose and 1.73 – 2.08g of stachyose in every 100g of beans (dry weight basis) (Table 6.3). In order to investigate the cultivar and region effect on the raffinose and stachyose content in raw beans, two-factor ANOVA was carried out (Table 6.4). Cultivar and region did not find to significantly affect the oligosaccharides content of raw beans.

Sanchez-Mata et al. (1998) determined raw navy beans contained 0.27 – 0.53g of raffinose and 1.65 – 2.49g of stachyose in every 100g of navy beans (dry weight basis) (also called white beans). This result was comparable with the results obtained in this project. No reports were found to study the cultivar and region effect on the raffinose and stachyose content raw beans.

Table 6.3 Oligosaccharides (raffinose and stachyose) content g / 100 g beans (dry weight basis) in raw beans for eight types of beans (bold) and other available bean samples (non-bold).

	Raffinose	Stachyose
A1	**0.47±0.030**	**1.97±0.11**
B1	**0.43±0.020**	**1.87±0.13**
C1	**0.43±0.041**	**1.73±0.17**
D1	**0.42±0.026**	**1.79±0.17**
A2	**0.45±0.013**	**1.87±0.12**
B2	**0.39±0.025**	**1.96±0.18**
C2	**0.42±0.054**	**2.08±0.27**
D2	**0.38±0.037**	**1.65±0.20**
B3	0.51±0.013	2.48±0.11
D6	0.38±0.021	2.15±0.16
D7	0.30±0.024	2.05±0.15
C5	0.35±0.058	1.88±0.13
C4	0.34±0.029	1.65±0.17
E5	0.42±0.025	2.06±0.13
F8	0.41±0.039	2.05±0.18
Ch09	0.41±0.047	2.06±0.04
Ch10	0.41±0.046	2.27±0.12

Values show mean of 3 replicates plus and minus the standard error of mean at 95% confidence interval.

Table 6.4 Two factors ANOVA P-value for amount of raffinose and stachyose in raw beans of eight types of beans.

	Raffinose	Stachyose
Cultivar	0.24	0.69
Region	0.31	0.61
Cultivar : Region	0.96	0.51

At 95% confidence interval

6.4.2 Exchange of oligosaccharides (raffinose and stachyose) between beans and sauce during canning

During canning, exchange of oligosaccharides (raffinose and stachyose) happened between beans and sauce. Canned sauce was centrifuged and supernatant was used to analyse the sugars by HPAEC-PAD. Figure 6.5 shows the representative HPAEC-PAD elution profiles of monosaccharides, disaccharides and oligosaccharide of canned sauce. 5 peaks have been identified which are internal standard fucose (peak 1- internal standard), glucose (peak 2), fructose (peak 3), raffinose (peak 5) and stachyose (peak 6). Peak 4 is un-identified peak because no standard for it was available. It is possible that peak 4 is sucrose but the resolution of the peak was poor. Small amounts of raffinose and stachyose (insert percentage) were released from beans into sauce after canning. According to a report by Oregon State University (Oregon State University, 2012), tomato paste contained no raffinose and stachyose. Therefore, all the raffinose and stachyose in the tomato sauce were believed to be leaching from beans.

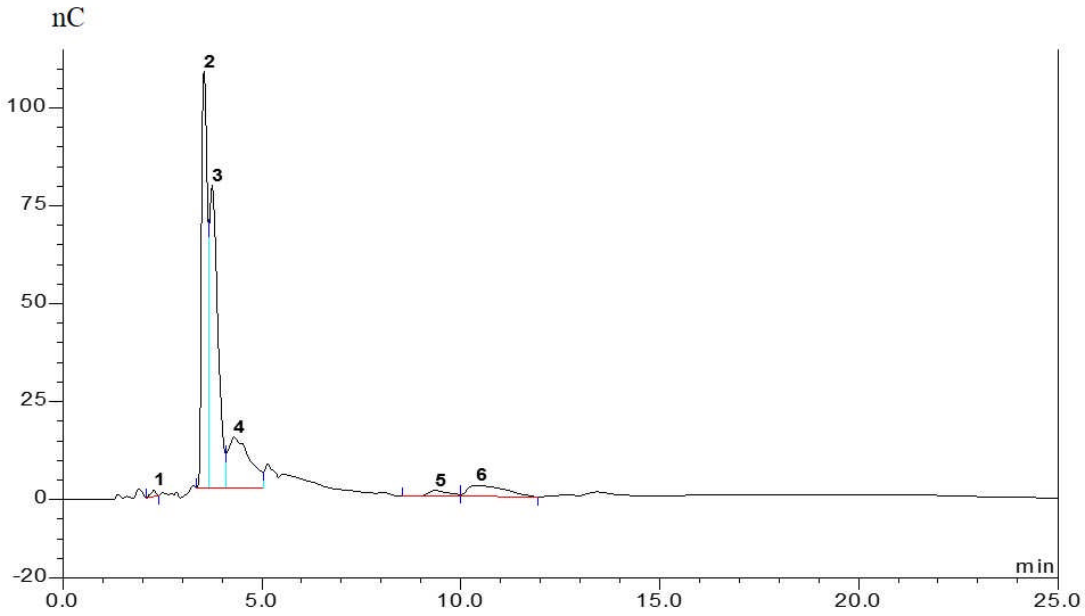

Figure 6.5. Representative HPAEC-PAD elution profiles of monosaccharides, disaccharides and oligosaccharide of canned sauce.

1 = fucose, 2 = glucose, 3 = fructose, 4= unidentified peak, 5 = raffinose, 6 = stachyose.

Table 6.5 shows the oligosaccharides (raffinose and stachyose) content (g/100g beans, dry weight basis) in canned sauce, and the raffinose and stachyose were leaching from beans into the sauce during canning. After canning, the beans leached 2% - 5% (0.01 – 0.02g) of raffinose and 2% (0.02 – 0.03g) of stachyose into the sauce in every 100g of beans (dry weight basis) (Table 6.5). Very small amounts of raffinose and stachyose were found in canned sauce.

No study had investigated the content of raffinose and stachyose in canned baked beans. But there were some studies reported the raffinose and stachyose content for beans cooked in water. Sat (2002) claimed that cooking (in water) would cause the sugar degradation, and pressure cooking would make the degradation more effective. There was 38% (dry weight basis) decrease of raffinose and 60% decrease of stachyose (dry weight basis) after pressure cooking (Sat and Keles, 2002). Similar decreasing was found by Oregon State University (Oregon State University, 2012), navy beans/common beans contained 0.3g raffinose and 1.5g stachyose for raw beans and 0.2g raffinose and 0.7g stachyose for cooked beans (in water). Therefore, it is believed that most of the oligosaccharides in the beans degraded to the

monosaccharides fructose, galactose and glucose. Therefore, small amount of raffinose and stachyose detected in the sauce was believed to be released from beans into sauce without degradation (2% - 5%, dry weight basis). However, the amount of raffinose and stachyose left in the canned beans after processing was not measured in this project.

Table 6.5. Oligosaccharides (raffinose and stachyose) in canned sauce as a percentage of content in raw beans g/100g (dry weight basis) for eight types of beans.

	Canned sauce (g/100g dry raw bean)	
	Raffinose	Stachyose
A1	0.01	0.02
B1	0.01	0.02
C1	0.01	0.02
D1	0.02	0.03
A2	0.01	0.02
B2	0.01	0.02
C2	0.02	0.03
D2	0.02	0.03

Values show mean of 3 replicates.

6.5 Storage effect on the properties of blanched and canned beans.

When the repeating experiments were carried out, it was accidently found that after storing the beans for 3 months at room temperature, the content of water soluble pectin for the blanched beans decreased significantly (Figure 6.6). All the beans samples were stored in the laboratory at room temperature (approximately 20°C) in an open package. Therefore, it inferred that storage conditions possibly have effect on the beans mechanical and chemical properties. Thus, more experiments were carried out to investigate the storage effect.

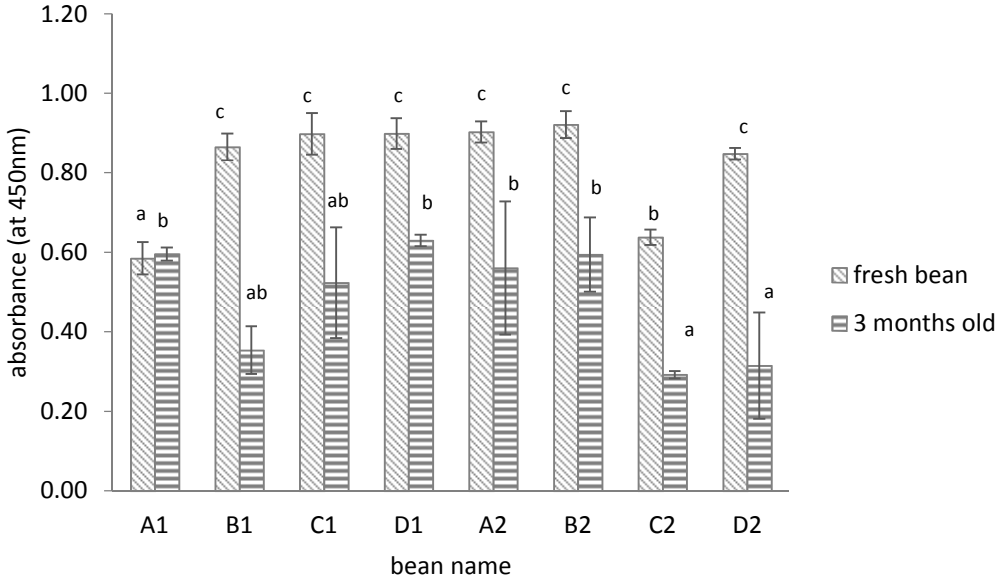

Figure 6.6 Soluble polysaccharides (un-methyl-esterified HG binding with antibody JIM5) leaching into the blanched liquid measured with ELISA method for eight types of beans in different storage time.

Values show mean of 3 replicates +/- standard error of means at 95% confidence interval. Letters a, b, c means within same column with the same superscript letters are not significantly different at p<0.05 levels.

In order to investigate the storage effect on the mechanical properties of blanched beans, cotyledon toughness was measured for 8 types of blanched beans. Figure 6.7 shows after stored for 6 months, all the beans were significantly firmer than the fresh beans. The toughness increased 4.8 – 6.8 times after storage. Therefore, it indicated that the storage had significant effect on the physical properties of blanched beans.

The storage effect was also found in canned beans firmness. Figure 6.8 shows the Kramer toughness of canned beans canning in water, brine and tomato sauce with different storage age. In Chapter 3, section 3.5, it has discussed that beans canning in tomato sauce were significantly firmer than canning in water and brine for the same age beans. However, after storage for another 1 year, beans canned in water and brine were significantly firmer than beans canning in tomato sauce (Figure 6.8). It also shows that 2 years old beans canned in water and brine were 1.6 times firmer than 1 year old beans. This may explain the results presented in chapter 3, where the

observed effect of cooking medium was opposite to what were found by Anzaldua-Morales and Brennan (1982). We found that fresh beans canned in tomato sauce were firmer than beans canned in brine and water, however Anzaldua-Morales and Brennan found the opposite results that beans canning in tomato sauce were softer than beans canning in brine. It is possible that different bean ages influence the conclusion for these two projects. Storage effect on the canned beans in tomato sauce was not able to investigate in this project because all the beans canned in tomato sauce were provided by Heinz to obtain the consistency. But it is interesting how the storage would have effect on the canned beans properties, so it can be further studied in the future.

Storage conditions effect was studied for raw legumes such as common bean, yellow pea, brown bean and lentils, and they all found during the storage, a higher temperature and higher moisture content would induce the hardening of the legumes and result in the hard-to-cook phenomenon (Shiga et al., 2004, Detoro, 1993, Iliadis, 2001, Stanley et al., 1990). Shiga et al. (2004) studied the navy beans (also called common beans) storage conditions effect on the mechanical and chemical properties. They found that under the accelerated condition (37°C, high moisture) beans were harder and showed loss of water-soluble pectins, and under mild condition (room temperature, high moisture) the beans showed a loss of Ara-rich polysaccharides. These results are comparable to the results found in this project that beans became significant firmer and showed loss of soluble un-methyl esterified HG after storage (Figure 6.7,Figure 6.8, Figure 6.8). It is of great interested that how the storage conditions influence the canned baked beans mechanical and chemical properties. It is also very important for the commercial application, because the un-appropriate storage condition may induce the hardening of the beans and increase the cooking time, and cost more energy which is environment and commercial benefit unfriendly.

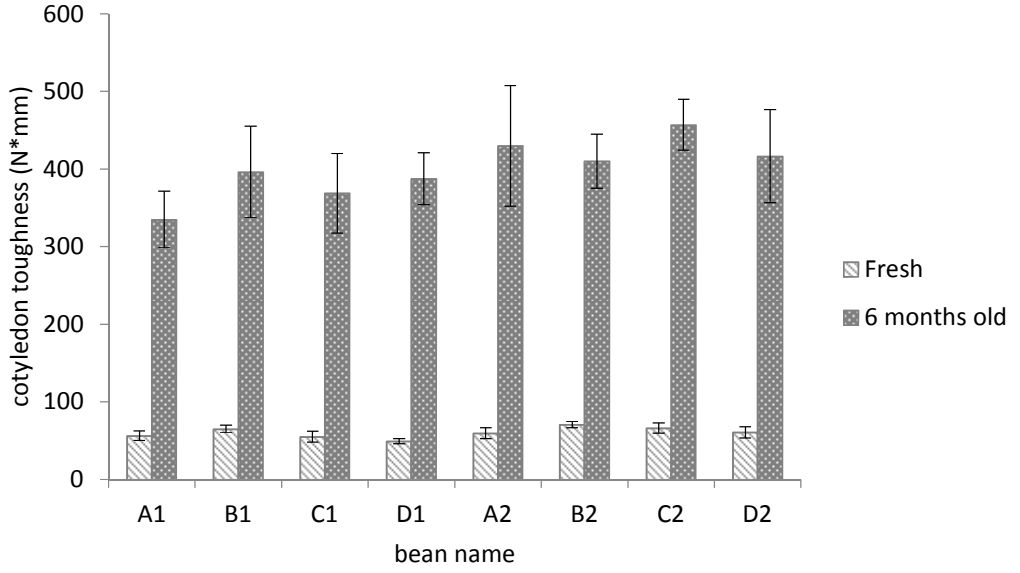

Figure 6.7. Cotyledon toughness for blanched beans after storing for different times.

Values show mean of 3 replicates +/- standard error of mean at 95% confidence interval.

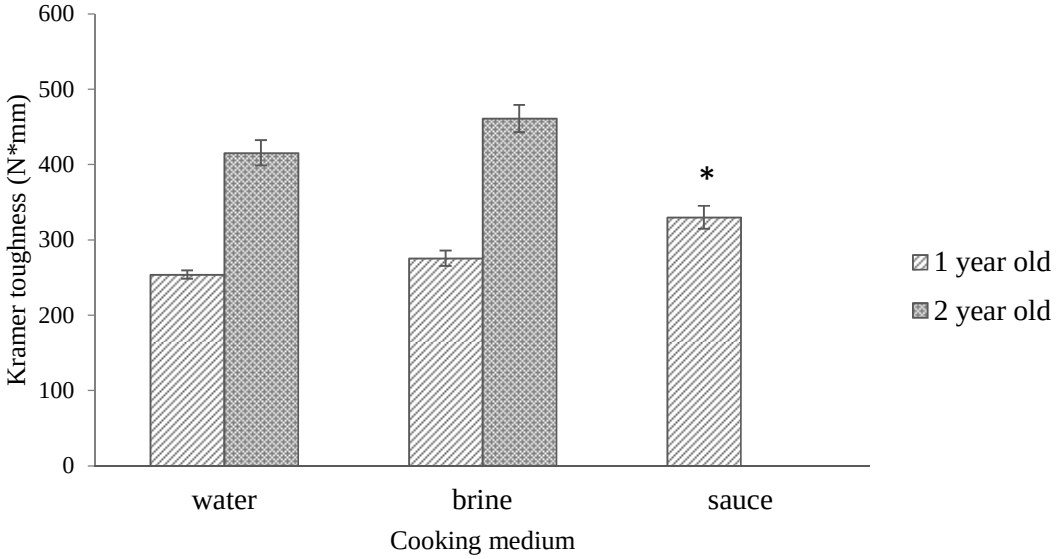

Figure 6.8. Kramer toughness of canned beans canning in different medium with different storage age.

Values show mean of 3 replicates +/- standard error of mean at 95% confidence interval. * means it significant different at p<0.05 levels.

6.6 Conclusion and discussion

Exchange of soluble pectin during canning was found between beans and sauce, the diffusion of bean polymers is likely to be affected by the sauce polymers. The diffusion directions showed differences between different pectins in canned beans. According to the comparison to the original sauce, non-methylesterified HG presumably originally from the beans increased in the sauce after canning by 15% (JIM5 epitope in sauce). Methylesterified HG presumably from tomato, decreased in the sauce after canning with a wide range (3% - 43% JIM7 epitope in sauce), and the results showed that the firm beans lost less and soft beans lost more. Galactan presumably from tomato, decreased in the sauce after canning with a large amount (64% LM5 epitope in sauce). And the galactan exchange from tomato sauce into beans may contribute to the firmer texture of canned beans compared to beans canning in other medium (water and brine). Arabinan presumably originally from the beans increased in the sauce after canning by 53% LM6 epitope in sauce, because large amount of arabinan detected in blanched medium as well, it indicated that arabinan is a very flexible and water soluble polysaccharides. In short, during canning process, soluble un-methylesterified HG and arabinan diffused from beans into tomato sauce, methylesterified HG and galactan diffused from the sauce into beans. The present study is the first to use antibody techniques to analyse bean soluble pectin, and it is therefore difficult to compare to the chemical analyses used in other studies. Antibodies are very specific probes that only binds to specific pectins, which is very different from other studies which determined the total pectin amounts. ELISA used in this project is a semi-quantitative method to analyse the soluble pectin in order to compare between different beans. Further investigation of the polymers that solubilise into the sauce is required, using a combination of chemical, biochemical and probably physical techniques with quantitative methods.

An average of 0.39g of raffinose and 2.07g of stachyose were detected in 100g of raw navy beans, however, only 2% of the original raffinose and stachyose content were detected in canned sauce. It is possible that majority of the raffinose and stachyose were degraded during thermal processing, or that these oligosaccharides are not solubilised into the sauce and remain in the bean. The raffinose and stachyose in the canned beans should be measured to give more information about the degradation of these oligosaccharides.

Beans canned in different medium (water, brine and tomato sauce) were also studied. Firmer texture was found when canning was done in tomato sauce than canning in brine or water. It indicated interactions between beans and sauce happened during canning. According to the results, non-methylesterified HG and arabinan, presumably originally from the beans increased in the sauce after canning, while methylesterified HG and galactan, presumably from tomato, decreased in the sauce after canning. The diffusion of bean polymers is likely to be affected by the sauce polymers, and may explain why beans canned in tomato sauce were firmer than canning in water or brine. Galactan has been found to contribute the firmness of pea cotyledon (McCartney et al., 2000), so it is possible that the decreasing content of galactan in the sauce diffused into the beans and contributed to the firmer texture. A small amount (15% increase in JIM5 epitope in sauce) of un-methylesterified HG diffused from the beans into sauce. Large increasing amount (up to 53% in LM6 epitope) of arabinan in the sauce compared to original sauce suggested the flexibility and water solubility of arabinan. The results from this study showed that arabinan-rich pectin from the beans is highly water soluble, leaching out during blanching and canning. After canning, both A1 (softest beans) and C2 (firmest beans) had relatively the highest amount of arabinan than the other beans. Using ELISA, there was a stronger effect of cultivar than region on the content of arabinan epitopes in the canning sauce, with cultivar 2 presenting significantly more than cultivar 1. However there was no correlation between soluble arabinan and bean firmness. Therefore, soluble arabinan cannot be used a predictor of bean texture. The present study is the first to use antibody techniques to analyse bean soluble pectin, and it is therefore difficult to compare to the chemical analyses used in other studies. Further work investigation of the polymers that solubilise into the sauce is required, using a combination of chemical, biochemical and probably physical techniques.

Kereliuk and Kozub (1995) investigated the chemical composition (including fibre, starch, protein) of beans from 4 cultivars grown in 3 regions (Ontario and 2 locations in Alberta). They found no significant differences amongst different cultivars, but found regions had significant effect on the composition of beans; beans from Ontario (in Kereliuk and Kozub's study is a softer bean) had lower amount of fibre and total sugar content than Alberta (firmer beans), but higher amount of starch content than Alberta. Regardless of the cultivar and region specific

properties, both the published and this research found that beans that contain higher fibre content tend to have lower amount of starch. Similar conclusions were also found in other reports, which believe that the cell wall enclosing the starch granules made the starch difficult to isolate and therefore result in higher fibre content (Hoover and Ratnayake, 2002, Hoover and Sosulski, 1985). The inclusion of non-digested starch in fibre values is an issue that can be resolved by the analysis of cell wall specific sugars in the fibre, in this study, fibre from beans with higher fibre content had a larger proportion of glucose, presumably from non-digested starch. Due to the difficulties associated with starch analysis, starch is not a reliable predictor of bean firmness.

Storage effect was found by accident, it was found after storage at RT in an open package for 6 months, firmness of the beans increased by 4.8 – 6.8 times. Soluble pectin (un-methylesterified HG) was found to decrease by 30% - 60% after 3 months storage. Therefore, it believed that storage conditions have significant effect on the canned beans mechanical and chemical properties. And it is very important for the industry to understanding the response of beans to the storage conditions, which may lead to improved efficiency in the food chain, while maintaining food quality at low cost.

Chapter 7
Correlations for chemical compositions and texture quality of canned baked beans

7.1 Introduction

The correlations between chemical composition and canning quality provide useful information for bean breeders. For example, the firmness of beans were found highly correlated with soluble pectin content (Lu and Chang, 1996). These information may provide manufactures a simple and rapid way to predict early. So that they can choose the bean which would produce the best canning quality beans, according to the commercial consideration as well as nutritional consideration. Therefore, in this chapter, correlations for physical/mechanical properties and chemical properties of navy beans were discussed.

In this chapter, the aims were: 1) Correlations were made among physical & mechanical properties, to find out how the physical and mechanical properties affect each other. 2) Correlations were made among chemical properties, to understand the relationships between the compositions of navy beans. 3) Correlations were made between mechanical properties and chemical properties, to investigate how the chemical properties determined the texture quality of canned baked beans.

Figure 7.1 shows the correlations will be discussed in this chapter.

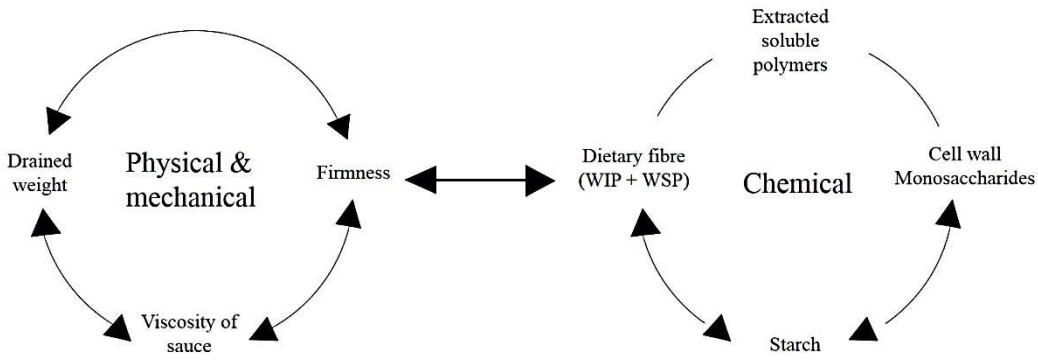

Figure 7.1 Correlations between properties of navy beans

7.2 Correlations for physical and mechanical properties

Industry prefers beans with higher imbibition for the sake of commercial consideration. So if the beans size or weight can predict the imbibition, it will give the easiest and most convenience prediction for the industry. Hence beans physical properties dry weight was correlated with water imbibition for blanched beans (water capacity) and canned beans (drained weight). Significant linear correlation was found for blanched beans but not canned beans (Table 7.1). However, Figure 7.2A shows that beans from region C (shaped x in the figure) dragged the line to two different directions, and C1 which was circled by dotted line may be the point made the relationship significant. Figure 7.2C also shows beans from region C (shaped x in the figure) dragged the line to two different directions. Therefore, beans from C were removed to correlate again. Table 7.1 shows that none of the correlations are significant for region A, B and D. In conclude, the beans size or weight cannot predict the imbibition of the beans for neither blanched or canned beans.

Table 7.1 Correlation coefficients (r value) for beans physical properties.

	Dry weight (8 types of beans)	Dry weight (exclude C1)
Water capacity	-.722*	.110
Drained weight	-.330	-.122

*. Correlation is significant at the 0.05 level.

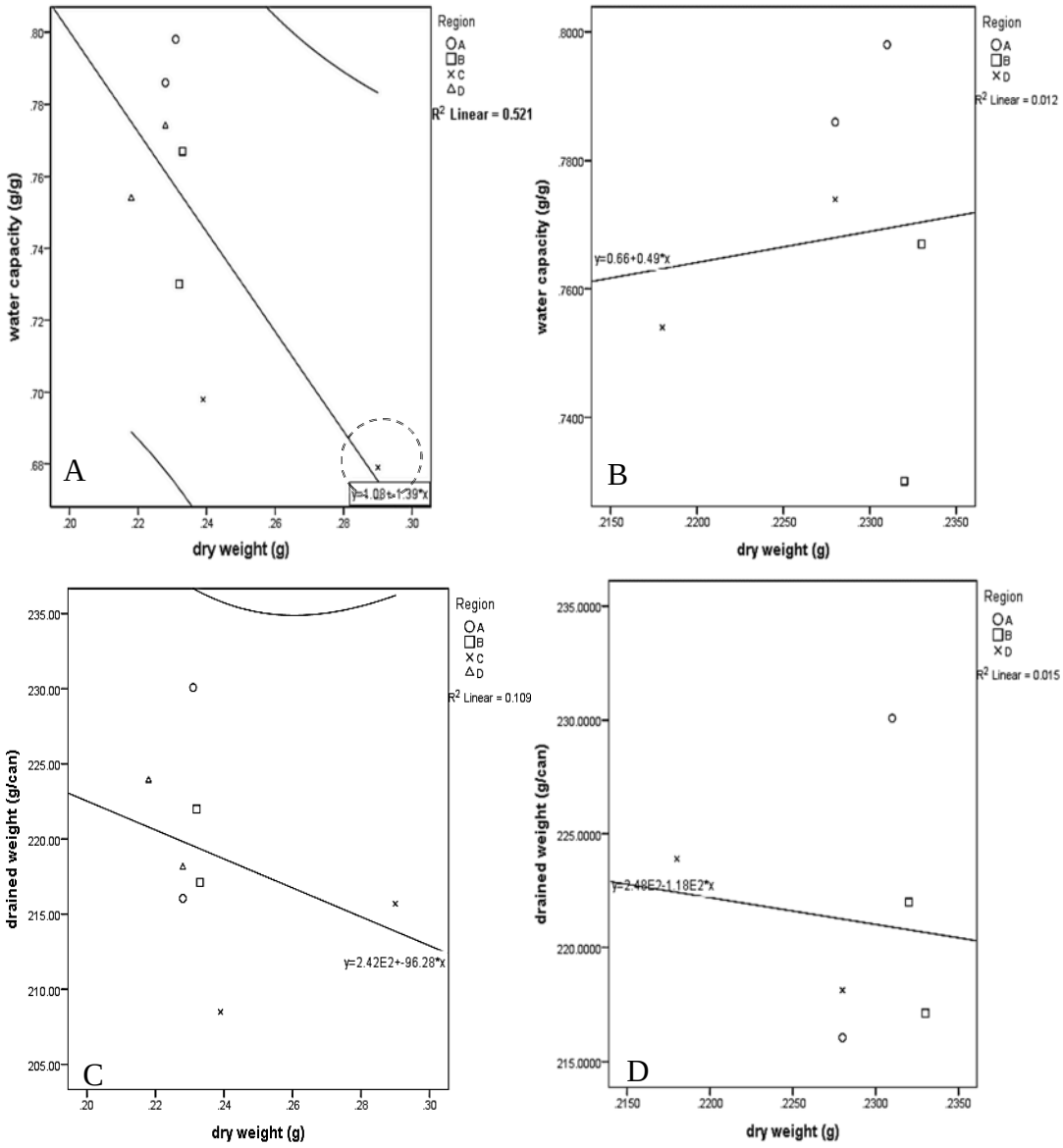

Figure 7.2 Relationship between dry weight and water capacity for eight types of beans (A) and eight types of beans exclude C1 (B) for blanched beans. Relationship between dry weight and drained weight for eight types of beans (C) and eight types of beans exclude C1 (D) for canned beans.

Values show mean of 20 replicates for dry weight and 3 replicates for water capacity and drained weight. Arcs represent the 95% confidential intervals. The point in circled dotted line is C1.

Table 7.2 shows the correlation between the firmness of beans (including blanched and canned beans) and drained weight & viscosity of canned beans. Drained weight was negatively correlated with the firmness of canned beans (r= -0.814*, r=-0.718*

and r=-0.752* for cotyledon, Kramer and Ottawa toughness respectively) although skin hardness was not significant (r= 0.600). The insignificant correlation for skin hardness properly caused by D1 which was circled by dotted lines in Figure 7.2A. It indicated that canned beans with firmer texture usually had lower water capacity. From what had found in Chapter 4, firm beans cells were attached to each other more tightly than soft beans, so that it is possible firm beans provided less space for the water to go through the cells, so that firm beans had less water capacity.

The viscosity of sauce was strongly negative correlated with beans firmness (r= -0.903*, r=-0.815* and r=-0.841* for cotyledon, Kramer and Ottawa toughness respectively) except skin hardness (r=-0.649). The insignificant correlation for canned skin hardness also caused by D1 which was circled by dotted lines in Figure 7.3 A. Because of the tightly attached cells, firmer beans may release less content into sauce or soaked in less content from the sauce than soft beans, so that the more content released from soft beans made the sauce more viscous. The viscosity also found to be positively significant correlate with viscosity of canned sauce (r= 0.768*), Therefore, firmer beans were found to have less drained weight and caused less viscous sauce after canning. Industry can predict the beans texture and sauce appearance according to the drained weight which is very easy and quick experiment to operate. Blanched beans mechanical properties did not find to affect the mechanical properties of canned baked beans.

There were reports studied on the correlations between the physical and mechanical properties of canned beans which were comparable with the results in this project. Lu and Chang (1996) claimed drained weight of canned beans was negatively correlated with the firmness of the canned beans and the viscosity of the canned sauce. They also found significant correlation between the firmness of canned beans and the splitting of the beans and the viscosity of the canned sauce.

Table 7.2 Correlation coefficients (r value) for beans physical and mechanical properties of blanched and canned beans.

		Drained weight	Viscosity of sauce
Canned	Drained weight	1	.768*
	Skin hardness	-.600	-.649
	Cotyledon toughness	-.814*	-.903**
	Kramer toughness	-.718*	-.815*
	Ottawa toughness	-.752*	-.841**
Blanched	Skin hardness	-.398	-.070
	Cotyledon toughness	-.260	-.039

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

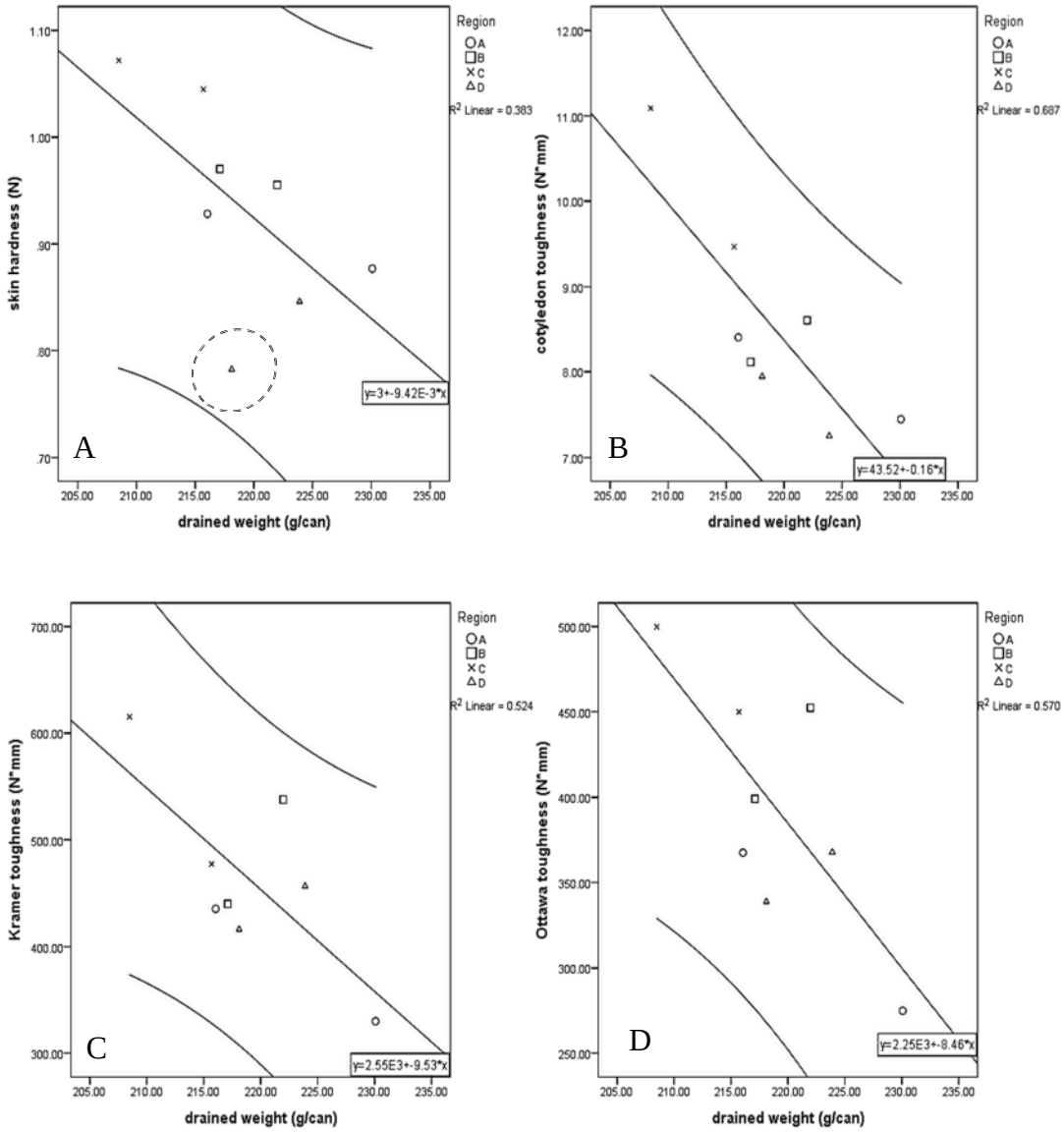

Figure 7.3. Relationship between drained weight and the firmness of the beans including skin hardness, cotyledon toughness, Kramer toughness and Ottawa toughness for eight types of canned beans.

Values show mean of 10 replicates for drained weight, skin hardness and cotyledon toughness, 3 replicates for Kramer and Ottawa toughness. Arcs represent the 95% confidential intervals. The point in circled dotted line is D1.

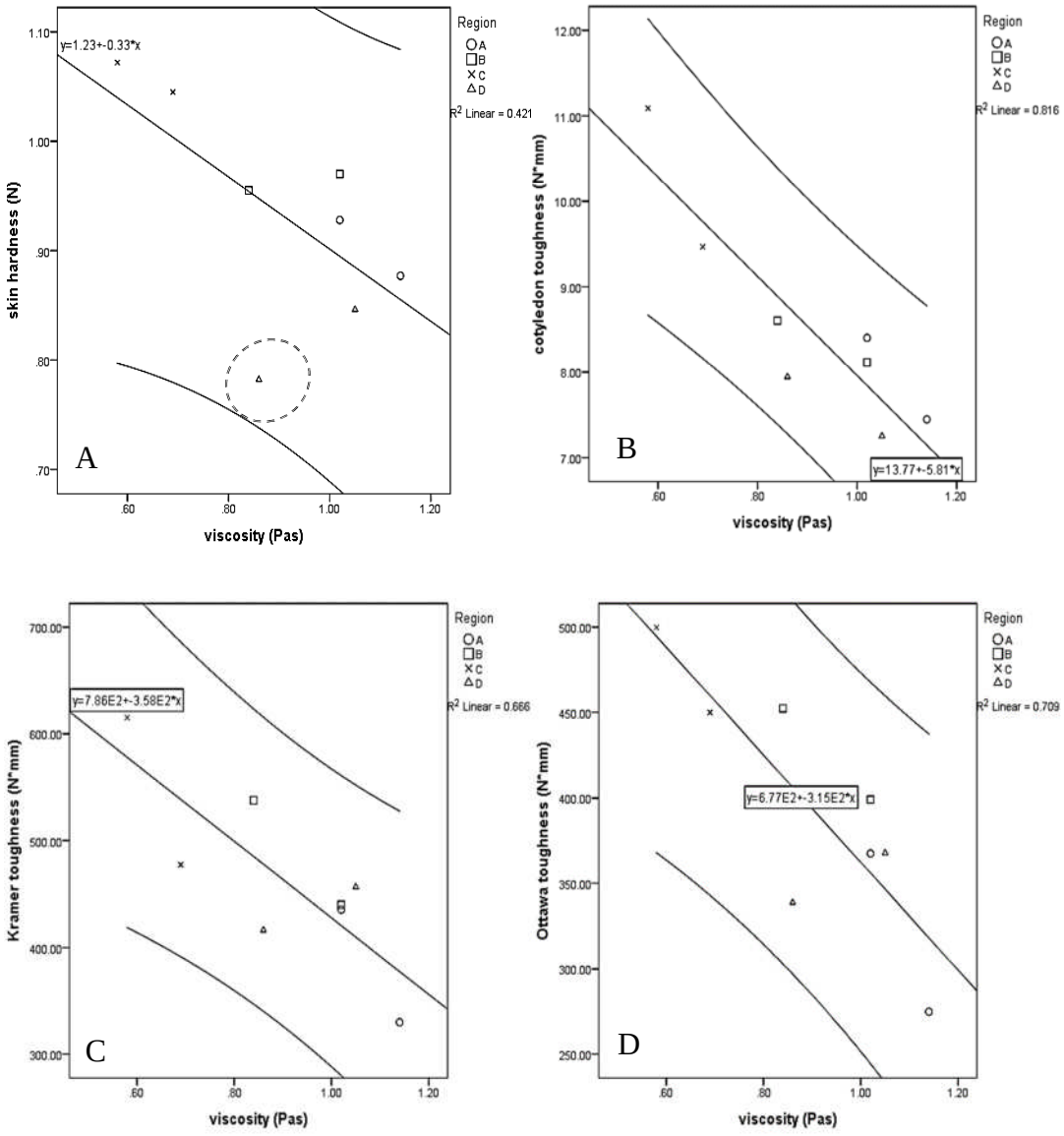

Figure 7.4. Relationship between viscosity and the firmness of the beans including skin hardness, cotyledon toughness, Kramer toughness and Ottawa toughness for eight types of canned beans.

Values show mean of 10 replicates for skin hardness and cotyledon toughness, 3 replicates for viscosity, Kramer and Ottawa toughness. Arcs represent the 95% confidential intervals. The point in circled dotted line is D1.

7.3 Correlations for chemical composition properties

Correlation for chemical composition would provide us as well as manufacture a good knowledge to understand the association among the navy beans components (Lu and Chang, 1996). Table 7.3 shows the correlations between starch content and the other chemical composition of canned beans. Starch content was found to significant correlate with WIP content (r = -0.747*) and glucose in WIP (r = -0.725*). Beans had higher content of fibre would had lower content of starch, and left more non-digested starch (which is the glucose in WIP) in WIP. Starch content was found to have a moderate correlation with total cell wall monosaccharides content (r = -0.485) and total monosaccharides content (r = -0.697), but not statistically significant. Table 7.3 also shows the correlation between starch content and WIP monosaccharides. Except strong linear correlation between starch and glucose in WIP. A moderate correlation was found between starch and arabinose (r = -0.673) and galactose (r = -0.660), but not statistically significant. The non-significant may due to the points too far away from the line, more replicates are needed to confirm the correlation. No correlation was found between starch content and xylose & mannose content.

Similar correlation between starch and fibre content was found from other report. Navy bean was found to have a lower proportion of starch compare to other legumes due to its higher fine fibre content (Hoover and Ratnayake, 2002). It was believed that the starch from legumes is difficult to isolate due to the presence of fibre which is derived from cell wall enclosing the starch granules (Hoover and Ratnayake, 2002, Hoover and Sosulski, 1985). Therefore, beans with high fibre content would cause lower detection of starch content, and left more non-digested starch content in the WIP.

Table 7.3 Correlation coefficients (r value) for starch content and other compositions of canned beans.

		Starch
	Dietary fibre	-.676
	WIP	-.747*
	WSP	.280
	Total CWM [A]	-.485
WIP	Arabinose	-.673
	Galactose	-.660
	Glucose	-.725*
	Xylose & Mannose	-.330
WSP	Arabinose	.603
	Galactose	.562
	Glucose	.231
	Xylose & Mannose	.618

A. Total CWM (cell wall monosaccharides) = arabinose + galactose + xylose & mannose.

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

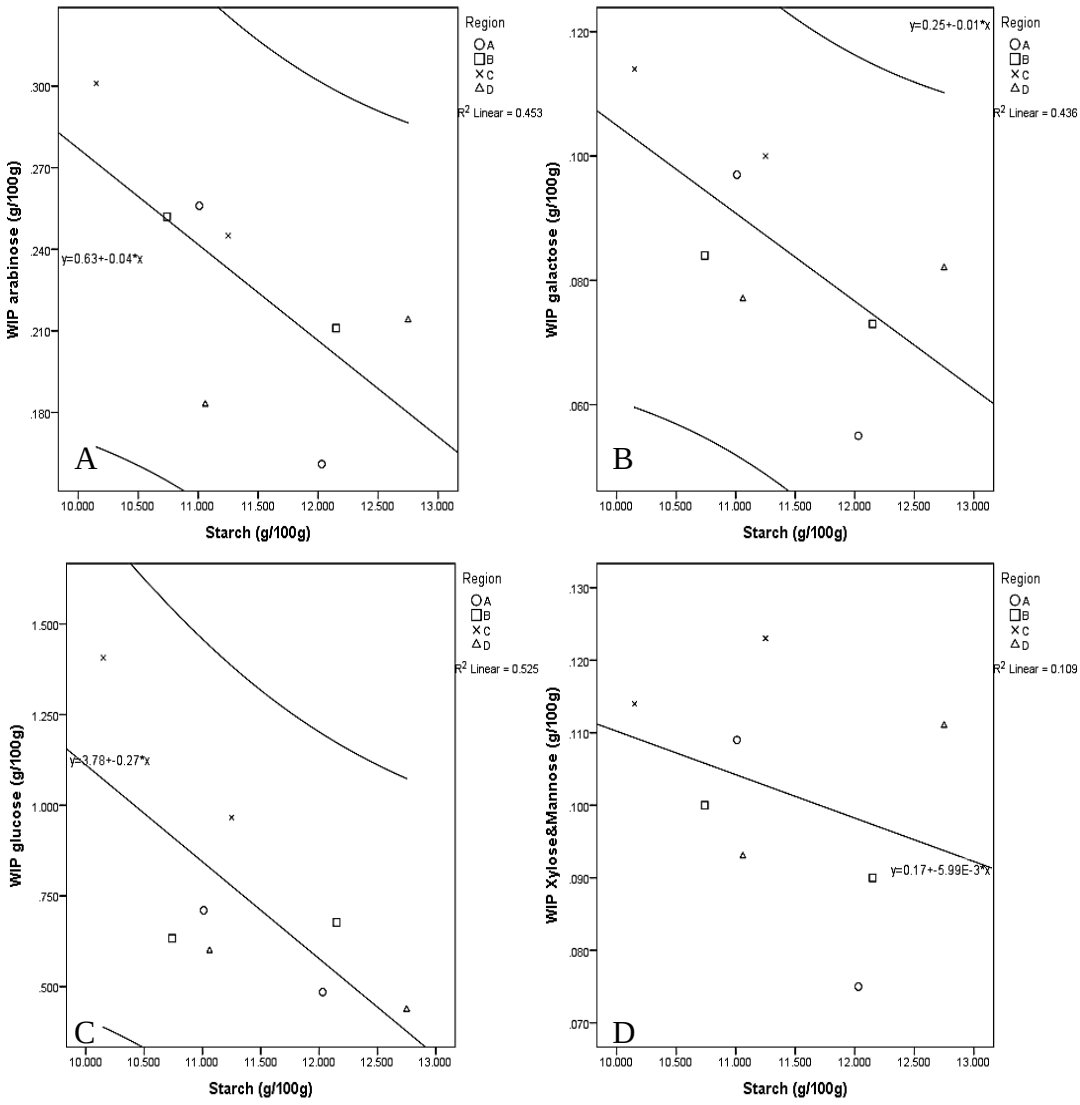

Figure 7.5 Relationship between starch content and WIP monosaccharides content: arabinose in WIP (A); galactose in WIP (B); glucose in WIP (C); and xylose & mannose in WIP (D).

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

7.4 Correlations between chemical properties and texture quality.

The correlation between chemical properties and canning quality is especially important for the manufacturers, they can get the information from the correlation about how the canning quality related to other properties, and maximum the commercial benefit.

7.4.1 Correlation between carbohydrate composition and texture quality of canned beans

In order to investigate the relationship between chemical composition and mechanical properties, the chemical composition include dietary fibre, WIP, WSP and starch content were correlated with beans firmness, drained weight and sauce viscosity (Table 7.4).

Beans firmness (including single skin hardness, single cotyledon toughness, whole batch beans Kramer toughness and Ottawa toughness) is significant positive correlated with WIP and dietary fibre content (Table 7.4). Beans firmness was not correlated with WSP content. Beans firmness was moderate correlated with starch content but not significant linear relationship with cotyledon toughness (r=-0.702).

Figure 7.6 shows the relationship between starch content and single beans cotyledon toughness. The non-significant relationship between starch content and cotyledon toughness may due to the points distant from the line, but more replicates are needed to confirm the correlation. The major composition of WIP was made of cellulose and hemicellulose (as discussed in chapter 5). The more WIP content gave the bean a stronger cell wall structure which harden the texture of the beans.

Drained weight of canned beans was highly negative correlated with WIP and dietary fibre content and highly positive correlated with starch content (Table 7.4). But drained weight did not have relationship with WSP content of canned beans. Therefore, high fibre content would reduce the water capacity of canned beans. It may due to the presence of fibre which is derived from cell wall provide an obstacle, and the obstacle obstructs the water absorption. High starch content would increase the water capacity of canned beans. Unlikely the fibre content, starch granules have

high ability of absorbing water, and swell a lot afterwards. Hence, more starch granules would help the beans absorb more water the increased the drained weight.

Table 7.4 Correlation coefficients (r value) between chemical composition and physical & mechanical properties.

		WIP	WSP	Dietary fibre	Starch
Bean firmness	Skin hardness	**.828***	.328	**.877****	-.509
	Cotyledon toughness	**.812***	.282	**.853****	-.700
	Kramer toughness	**.742***	.361	**.798***	-.372
	Ottawa toughness	**.854****	.326	**.902****	-.424
Drained weight		**-.900****	.223	**-.855****	**.835****
Viscosity of sauce		-.702	-.348	**-.755***	.545

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

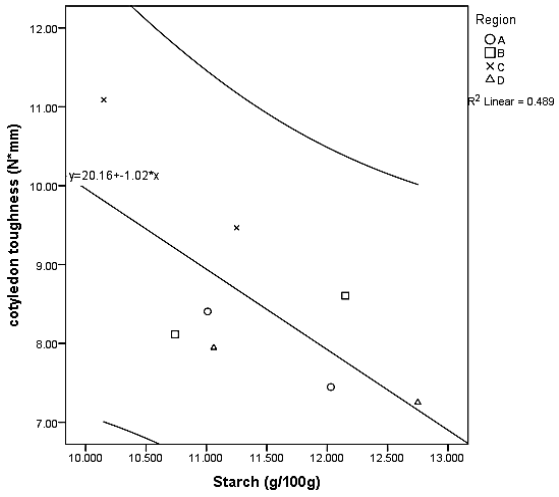

Figure 7.6 Relationship between the starch content and the cotyledon toughness for eight types of canned beans.

Values show mean of 10 replicates. Arcs represent the 95% confidential intervals.

Figure 7.7 shows the relationship between sauce viscosity and chemical content. Only significant relationship was found between sauce viscosity and dietary fibre. There was a negative correlation (r=- 0.755*) that the higher amount of dietary fibre the beans contained, the less viscous the sauce would be. It may because the present of fibre which derived from cell wall provide an obstacle and less chemical content release from the beans into the sauce and decreased the viscosity of the sauce. There was a moderate negative relationship between WIP and sauce viscosity (r=- 0.702) but not statistic significant. Figure 7.7 A indicates the non-significant may due to B2, which was circled by dotted lines. B2 was distant from the line and reduce the coefficients r value, more replicates are needed to confirm the correlation. The sauce viscosity did not have any relationship with WSP content (Figure 7.7 B), and have a very weak relationship with starch content (Figure 7.7 D, r=0.545). Hence, although starch content is possibly increase the sauce viscosity, but because viscosity is significantly influenced by cell wall content. So the relationship between starch and sauce viscosity was very weak. However, the viscosity of sauce is not due to single constituent but numbers of factors worked together (Costa et al., 1993). saponins (Topping et al., 1984) and the composition of the protein (Sirtori et al., 1979, Gibney, 1982) have all been suggested as the causative factors to effect the viscosity of the sauce.

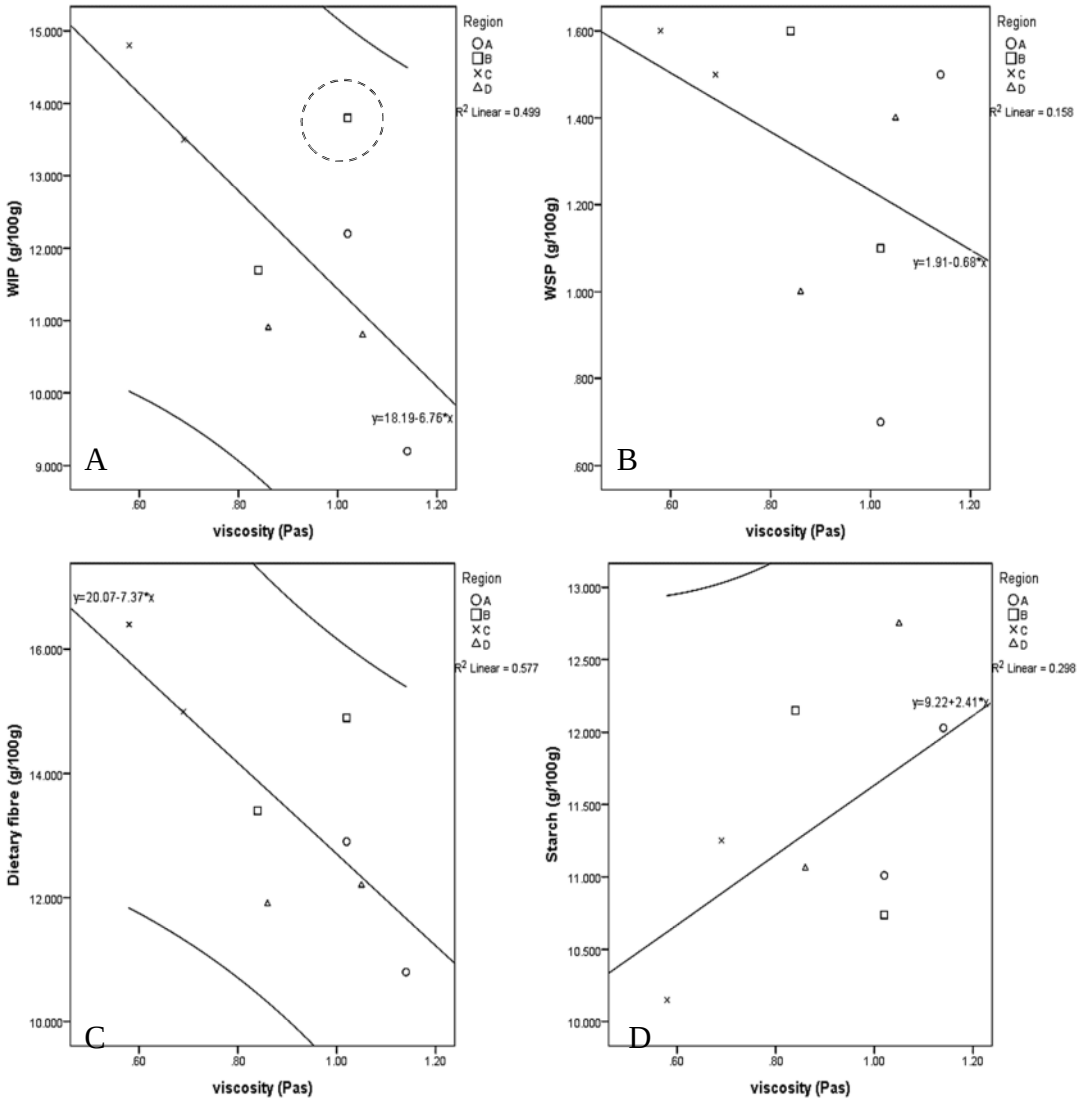

Figure 7.7. Relationship between the viscosity and the WIP content (A); WSP content (B); dietary fibre content (C) and starch content (D).

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals. The point in circled dotted line is B2.

7.4.2 Correlation between cell wall monosaccharides and texture quality

As discussed in chapter 5, dietary fibre was hydrolysed into monosaccharides, which were derived from cell wall neutral sugar. Table 7.5 shows the correlations between cell wall monosaccharides and the physical & mechanical properties of canned beans. For WIP composition, arabinose of WIP shows linear correlation with all physical and mechanical properties except sauce viscosity. Figure 7.8 A shows that the points are linear distribution however distant from the line, so that the

relationship is not significant. Galactose of WIP shows linear correlation with all physical and mechanical properties except skin hardness. There was a moderate linear relationship (r=0.679), but because the points are distant from the line so that the relationship is not significant (Figure 7.8 B). Xylose & mannose only had significant correlation with drained weight, but had weak correlation with beans firmness and sauce viscosity.

Figure 7.9 shows that the xylose & mannose of WIP are relative weak correlated with beans firmness and sauce viscosity although they do not have significant linear relationship. The non-significant correlation between xylose & mannose of WIP and beans firmness is likely due to some points are distant from the line but still weakly linear distribution (Figure 7.9, A B C D). The correlation between xylose & mannose of WIP and sauce viscosity is likely not a linear correlation (Figure 7.9, E). Therefore, beans with higher drained weight would have less amount of arabinose, galactose, glucose and xylose & mannose in WIP; firmer beans would have higher amount of arabinose, galactose and glucose in WIP; higher amount of arabinose, galactose and glucose in WIP would cause less viscous sauce.

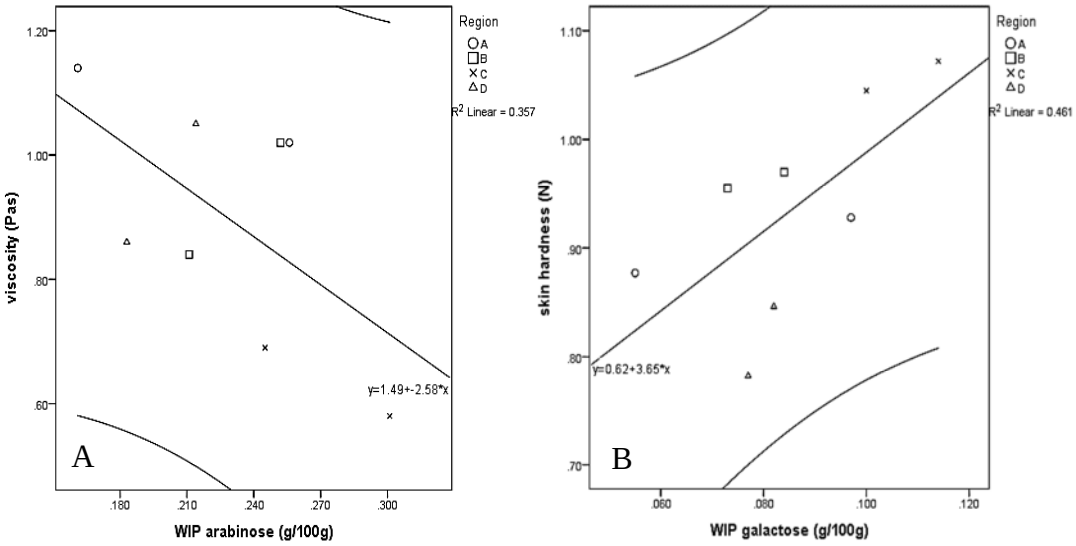

Figure 7.8 Relationship between the WIP composition and physical & mechanical properties. Arabinose of WIP *vs.* viscosity (A), galactose of WIP *vs.* skin hardness (B).

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

Table 7.5. Correlation coefficients (r value) between cell wall monosaccharides and physical and mechanical properties.

		WIP			WSP			Total CWM [A]
		Arabinose	Galactose	Xylose & Mannose	Arabinose	Galactose	Xylose & Mannose	
Beans firmness	Skin hardness	.789*	.679	.541	-.130	.142	.095	**.828***
	Cotyledon toughness	.786*	.804*	.540	-.213	.021	-.002	**.825***
	Kramer toughness	.750*	.722*	.549	-.244	.161	.161	**.789***
	Ottawa toughness	.793*	.758*	.644	-.281	.171	.150	**.842****
	Drained weight	-.881**	-.929**	-.711*	.643	.454	.471	**-.792***
	Viscosity of sauce	-.597	-.720*	-.560	.137	-.155	-.144	**-.718***

A. Total CWM (cell wall monosaccharides) = arabinose + galactose + xylose & mannose

B. Total detected monosaccharides from hydrolysed dietary fibre = cell wall polysaccharides + glucose

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level

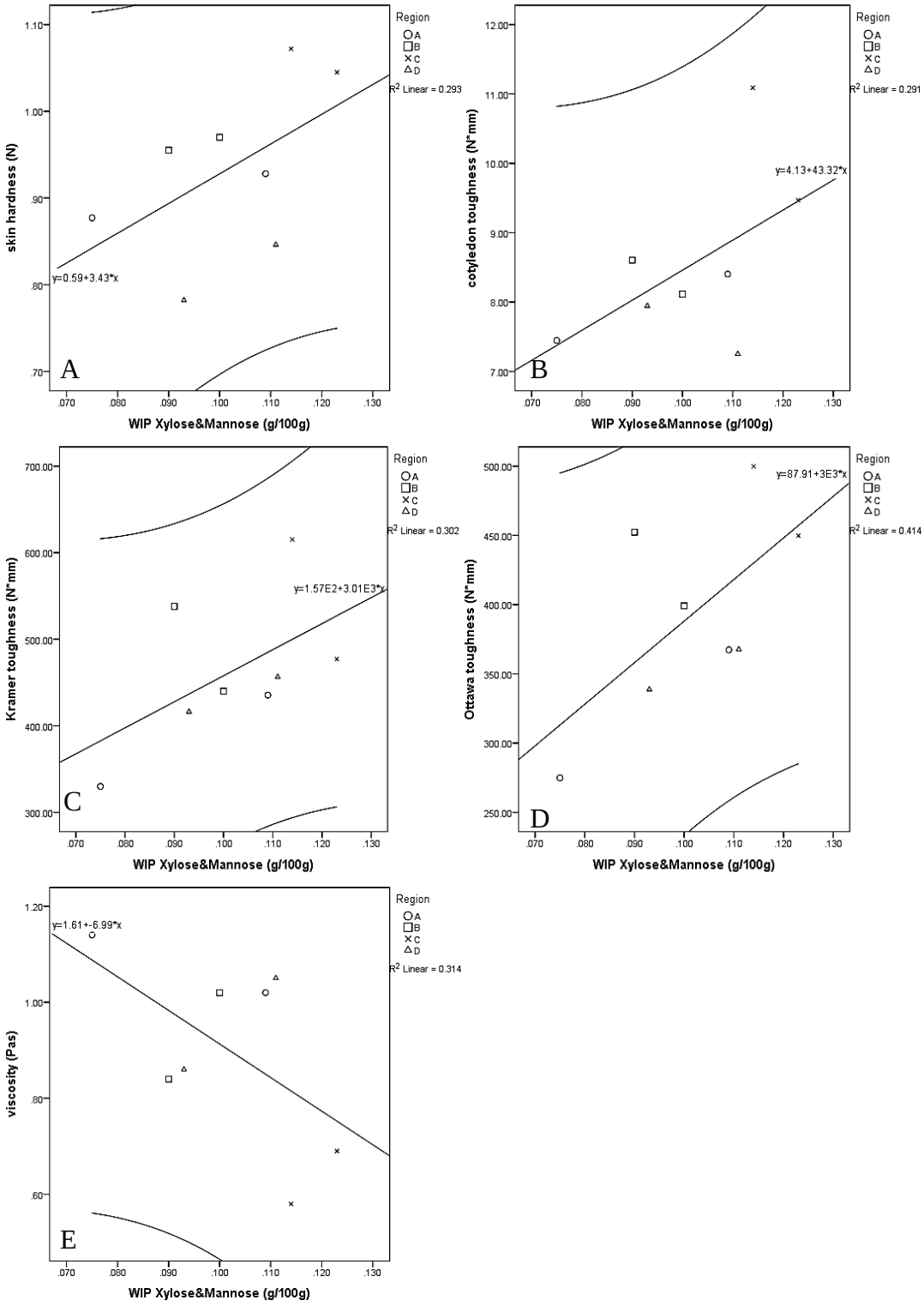

Figure 7.9 Relationship between the xylose & mannose of WIP and beans firmness including skin hardness (A), cotyledon toughness (B), Kramer toughness (C), Ottawa toughness (D), and sauce viscosity (E).

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

For WSP composition, no significant relationships were found for any physical or mechanical properties (Table 7.5). But the arabinose of WSP shows moderate linear correlation with drained weight (Figure 7.10), but not significant. Hence, the composition of WSP is likely not influence the beans physical and mechanical properties.

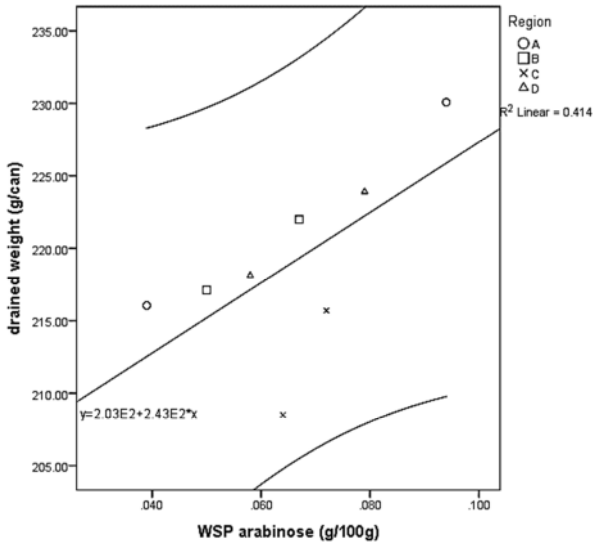

Figure 7.10. Relationship between the arabinose of WSP and drained weight of canned beans.

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

For total cell wall monosaccharides (CWM), it was highly correlated with all the beans physical and mechanical properties (Table 7.5). Figure 7.11shows relationship between the total cell wall monosaccharides and beans physical & mechanical properties. It indicated that higher amount of cell wall monosaccharides that beans contained, beans would had the lower drained weight, firmer beans (including skin hardness, cotyledon toughness, Kramer toughness and Ottawa toughness) and lower viscous sauce. Therefore, it indicated that the cell wall neural sugar played a very important role related the beans physical and mechanical properties.

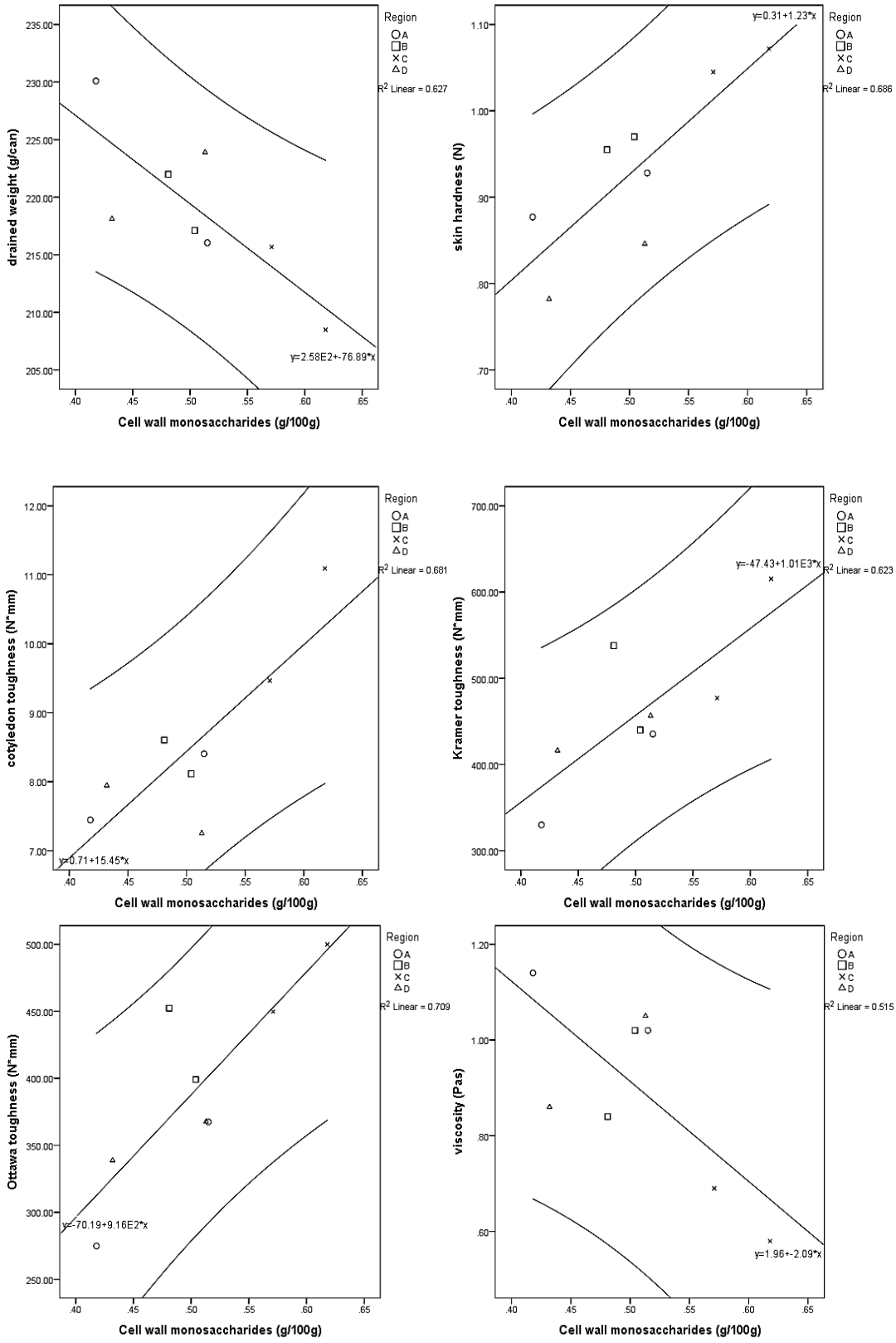

Figure 7.11 Relationship between total cell wall monosaccharides and physical & mechanical properties of canned beans.

Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

7.4.3 Correlation between soluble pectin content and canning quality

Some of the water soluble pectin left in the dietary fibre as WSP. And some were leaching into the cooking medium during cooking. Table 7.6 shows the correlation between soluble pectin and physical & mechanical properties of blanched beans. No relationships were found between blanched beans soluble polysaccharides content and the water capacity or firmness of blanched beans. Therefore, blanched beans soluble pectin cannot be the predictor for the canning quality of canned beans.

Table 7.6 Correlation coefficients (r value) between soluble polysaccharides and physical & mechanical properties of blanched beans.

	JIM5	JIM7	LM5	LM6
Water capacity	-.060	.144	.215	.014
skin hardness	.113	-.011	.185	.236
cotyledon toughness	-.029	.275	.253	.315

*. Correlation is significant at the 0.05 level.

Table 7.7 shows the correlation between the soluble pectin in the sauce (including soluble pectin from beans and pectin in tomato sauce) and the physical & mechanical properties of canned beans. No relationships were found for un-methyl-esterified HG (labelled with JIM5), galactan (labelled with LM5), or arabinan (labelled with LM6). Methyl-esterified HG (labelled with JIM7) is likely to have a better correlation compared to the other polysaccharides, however, only drained weight is significantly negatively correlated with JIM7 (r=-0.724*, Figure 7.12). Therefore, canned beans which had higher drained weight would have less methyl-esterified HG in the sauce. As discussed in chapter 6, after processing, methylesterified HG transported from sauce into beans. Table 7.7 shows beans firmness had a moderate positive correlation with JIM7, although it is not significant. Chapter 6 also indicated that the firmest beans had highest amount of JIM7 in the sauce and the softest beans had smallest amount of JIM7 in sauce. Therefore, it is possible that firmer beans are harder to soaked in the

methylesterified HG, and firmness was negative correlated with drained weight, that explained why the JIM7 negatively correlated with drained weight of canned beans.

Table 7.7 Correlation coefficients (r value) between soluble polysaccharides in the sauce and physical & mechanical properties of canned beans.

		JIM5	JIM7	LM5	LM6
Beans firmness	skin hardness	-.313	.419	-.191	.002
	cotyledon toughness	.167	.577	-.357	-.020
	Kramer toughness	.051	.653	-.374	.302
	Ottawa toughness	-.151	.588	-.486	.126
	Drained weight	-.081	**-.724***	.537	-.063
	viscosity	-.215	-.501	.573	.186

*. Correlation is significant at the 0.05 level.

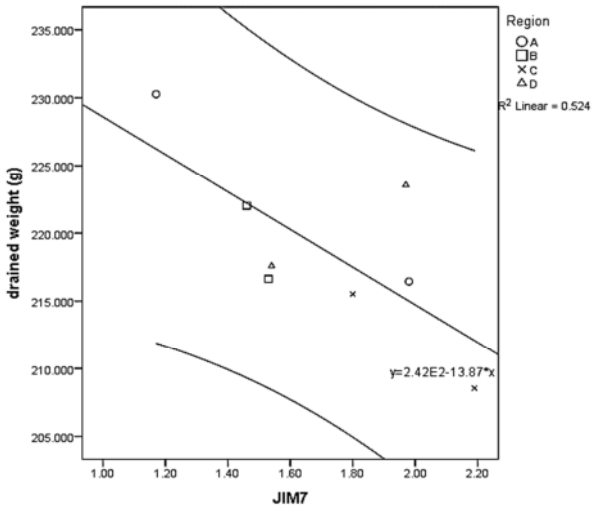

Figure 7.12. Relationship between methyl esterified HG (labelled with JIM7) and the drained weight for eight types of canned beans.
Values show mean of 3 replicates. Arcs represent the 95% confidential intervals.

There was no study working on the correlation between water soluble pectin and texture of the blanched beans. But there were some reports studied on the correlation between soluble pectin and texture of canned beans. Wang et al. (1988) claimed that there was a negative relationship between soluble pectin and firmness for canned beans. However, this result was not comparable to the results in this project. Wang et al. (1988) determined the soluble pectin in canned beans using Na-phosphate buffer to extract the total pectin. In this project, canned beans soluble pectin were determined using anti-pectin probe from the canned sauce (including soluble pectin from the beans and from the sauce) for four types of specific pectin. Hence, it may have significant correlation between beans firmness and total soluble pectin in the canned beans (Wang et al., 1988), but it was not necessary any of the specific pectin (in this project were HG, galactan, arabinan) had significant correlation with firmness of the beans.

There were also some reports studied on the relationship between the texture of canned beans and soluble content of raw beans. Wang et al. (1988) determined the soluble content of raw beans using Na-phosphate buffer to extract the pectin, and correlated with canned beans firmness. They found significant negative correlation between soluble pectin of raw beans and firmness of canned beans. Lu and Chang (1996) reported that soluble pectin of raw beans was negatively correlated with firmness and overall acceptance of canned beans. The data for the soluble pectin in the raw beans is not available in this project. But it is interesting for the future work because it can be a good predictor if there is a relationship.

Table 7.8 shows the relationship between the extracted oligosaccharides content in raw beans and physical & mechanical properties of canned beans. No significant correlations were found between oligosaccharides content in raw beans and any of the physical & mechanical properties of canned beans. Therefore, the oligosaccharides content in the raw beans did not find to influence the canning quality of canned baked beans, and cannot predict the properties of the quality of canned beans.

Table 7.8 Correlation between oligosaccharides content in raw beans and physical & mechanical properties of canned beans

	Raffinose	Stachyose
Drained weight	.239	-.272
Skin hardness	.118	.445
Cotyledon toughness	.123	.486
Kramer toughness	-.275	.243
Ottawa toughness	-.248	.191
Viscosity of sauce	.015	-.176

7.5 Conclusion and discussion

Among the physical and mechanical properties, bean weight was not found to correlate with the water capacity for either blanched beans or canned beans. The firmness of blanched beans were not found to correlate with any physical or mechanical properties. However, the firmness of canned beans (including single bean firmness and whole batch of beans firmness) was highly correlated with drained weight and viscosity of the sauce. It indicated that firmer beans had less drained weight and less viscous sauce in the final production. The microstructure of the cells may be the determinant of this relationship, firmer beans displayed more tightly attached cells which prevented the water absorption and soluble polymers exchange, consequently decreased the water capacity and sauce viscosity. Therefore, the industry should avoid select the beans which are too firm, because it will result in thin sauce which would cause customer complaints and poor water capacity would increase the original cost, decreased the commercial benefit.

Among the chemical properties, starch content was found negatively correlated with the WIP content. Beans contained higher amount of WIP were harder to digest with amylase, because the WIP which is derived from cell wall may be enclosing the starch granules, providing a barrier for the digestion of starch. Thereafter, more non-digested starch left in the WIP, which explained the highly negative correlation between starch content and the glucose content in WIP.

The correlation between the carbohydrate composition and physical & mechanical properties indicated that firmer beans contain higher amount of dietary fibre content and WIP content. Higher content of dietary fibre content would result in less viscous sauce. Drained weight of canned beans was negative correlated with dietary fibre and WIP content, and positive correlated with starch content.

Cell wall monosaccharides content was found to be highly correlated with canned beans physical and mechanical properties. Firmer beans were found to contain higher amount of total cell wall monosaccharides, arabinose in WIP and galactose in WIP. Drained weight was negative correlated with total cell wall monosaccharides and arabinose, galactose and xylose & mannose in WIP. Higher amount of total cell wall monosaccharides and galactose in WIP would cause less viscous sauce. However, WSP content was not found to have any correlation with the texture quality of canned beans.

The four specific soluble pectin in the canned beans including un-methylesterified HG, methylesterified HG, galactan and arabinan were not found to correlate with the firmness of canned beans, although many reports have claimed the total soluble pectin content of raw beans was negatively correlated with the firmness of canned beans (Lu and Chang, 1996, Wang et al., 1988). Raffinose and stachyose of raw beans were not correlated with any texture quality of canned beans.

From the correlations found above, drained weight showed a good characteristic to be a predictor of mechanical properties. It highly correlated with the firmness of beans, the dietary fibre content, the WIP content, the starch content, as well as the soluble pectin content in the canned beans. Drained weight is very easy to operate and obtain the result. It is very easy, fast, convenient and cheap way for the industry to briefly predict the canning quality of the canned baked beans.

Chapter 8
Conclusion

In this study, the effect of cultivar and region on different properties were investigated. Figure 8. 1 shows the multivariate analysis of these properties, including mechanical, structural and chemical properties of navy beans. Comprehensively, beans from C and D have relatively consistence properties between cultivars than beans from A and B. So that, region had larger effect on overall properties than cultivar for beans from C and D. however, cultivar had larger effect than region for beans from A and B. That may be the reason that significant interactions happened between region and cultivar. Because the selection of region is difficult because of lack of land available. So that the selection of the cultivar for commercial cultivation has to be considered comprehensively, and using information on quality parameters could select the cultivars/region combinations that will provide optimal quality. In an ideal world, the industry would select a bean that does not vary very much between regions, to provide the best consistency across the growers. From this perspective, cultivar 2 varies less when grown in different regions, compared to cultivar 1 (Figure 8. 1). Processors already optimise the processing conditions depending on what beans are available. Different products require different starting properties to produce the final quality. For example, industry may use firmer beans for larger packs that need longer processing, in this case, cultivar 2 may be preferred. And softer beans from cultivar 1 maybe preferred for small packs. New packaging and processing techniques may also require different starting beans. Therefore, the aim of the project was to increase understanding of the cultivar and region effects on mechanical properties of the beans, to provide processors with better understanding of raw material before process optimisation.

Overall, drained weight was found to be a very good predictor for the industry to predict the canning quality of canned baked beans. This is because it was found to be highly correlated with mechanical properties and chemical properties. Drained weight had significant correlations with beans firmness ($r = -0.75^*$), sauce viscosity ($r = 0.77^*$), dietary fibre ($r = -0.86^{**}$), WIP ($r = -0.9^{**}$), starch ($r = 0.84^{**}$), total

cell wall neutral monosaccharides (r = -0.79*), arabinose (r = -0.88**) in WIP, galactose (r = -0.93**) in WIP, xylose and mannose (r = -0.71*) in WIP and soluble methylesterified HG (r = -0.72*) in tomato sauce. It indicated that under the same canning procedure, higher drained weight would have softer beans, thicker sauce, less fibre content and WIP content, higher amount of starch in the beans, less amount of cell wall neutral monosaccharides (including arabinose, galactose and xylose & mannose in WIP), and less amount of methylesterified HG in the tomato sauce. Drained weight is also very easy to obtain, with simple operation and non-destructive. Therefore, drained weight can be the priority choice to briefly predict the canning quality of the canned baked beans for the industry.

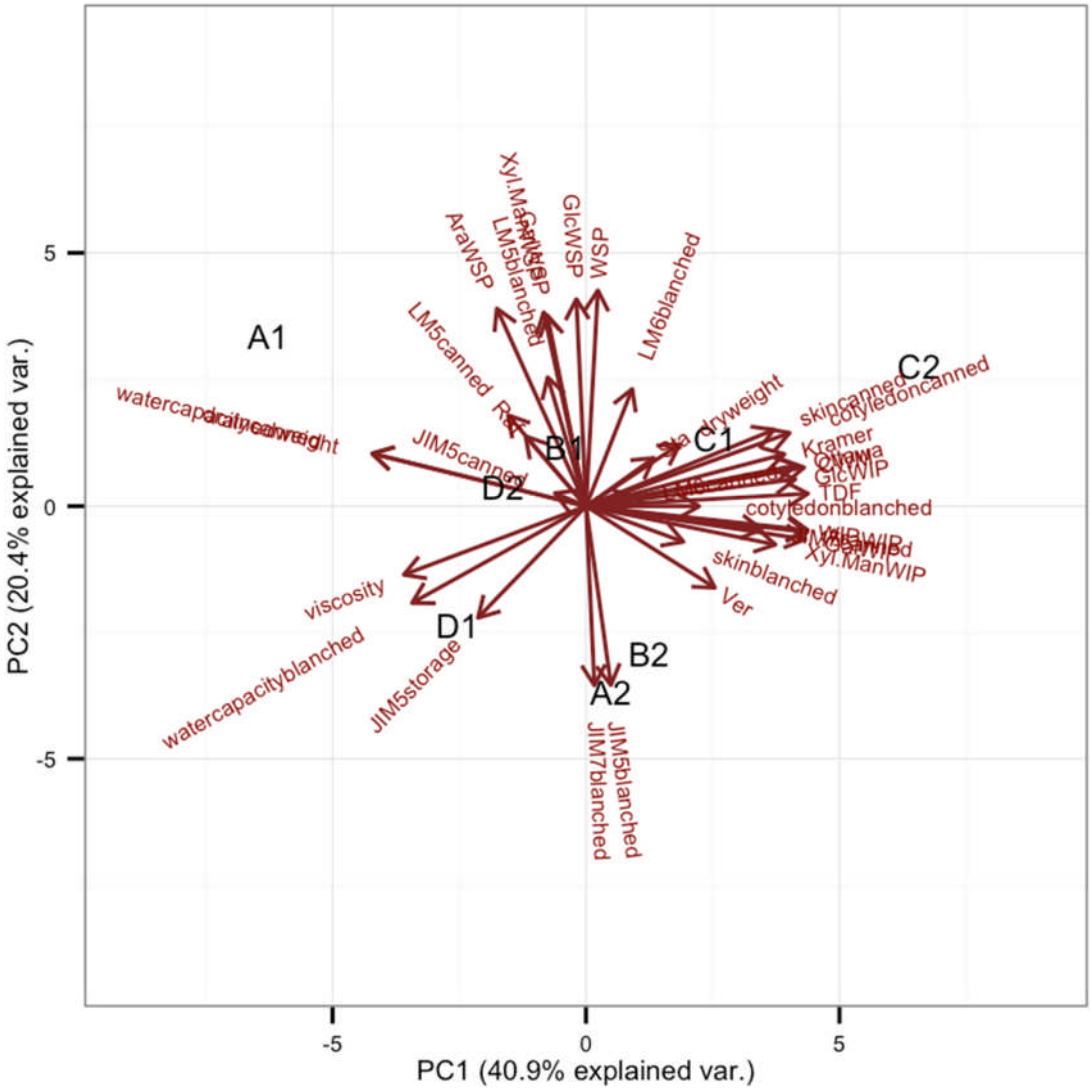

Figure 8. 1 Multivariate analysis of different properties of eight types of beans.

Mechanical properties

Cultivar and region were found to have significant effect on the firmness of canned beans with large interactions, indicating that cultivars respond differently depending on the regions. Using firmness of a whole batch of beans (Kramer toughness) as a quality parameter, both region and cultivar affected mechanical properties, with beans grown in C and B having firmer texture than beans grown in D and A (Figure 8. 1), and region had a stronger effect than cultivar, for example A2 is softer than C1. As discussed in chapter 3, firmness of a whole batch of beans provided more discriminative measure of bean firmness, compared to single bean measurements. This means that analysing the firmness of a whole batch of beans provides a more detailed representation of the cultivar/region effects.

Structural properties

The mechanical properties of the beans were directly influenced by the structure of the cells. Firmer beans were found to have thicker cell walls, which was believed to give more support and contributed to the beans' rigidity. Beans softened dramatically after processing, but did not lose their shape. The microstructure of cells changed significantly. As shown in Figure 8.2, bean cells were hexagonal shape before canning, cells adhered together tightly through the middle lamella. The middle lamella is a pectin-rich layer that joins together the primary walls of adjacent cells. The triangle shape spaces binding (JIM5 and JIM7 epitopes) between cells which are called intercellular spaces were clearly presented in the tissue before canning. After canning, the cell shape changed from hexagonal to round. The cells swelled up to 30% in volume compared to raw beans, the starch granules expanded up to two times lager and filled in the cells. The canning process loosened the middle lamella and the primary cell wall, the triangle shape spaces binding were not visible between most of the cells, replaced with large spaces between cells filled with pectin. The firm beans were found have tighter cell adhesion than soft beans, but all the beans cells were still strong enough to keep the cell intact, despite large intracellular pressure given by the swollen starch granules. The cell adhesion was believed to be largely attributed by the calcium cross linked homogalacturonan

which was intensely bond by JIM5. Therefore , the microstructure of the cell wall influences mechanical properties. Pectin may play a ‘super glue’ role between cells, a small amount of the pectins in the middle lamella and intercellular spaces adhered the cells tightly together and provided structure to the beans. After the ‘glue’ loosened during canning, beans texture soften dramatically. However, the differences between different beans cannot be visualised using microscopy. The differences observed between beans were subtler compared to the mechanical and structural changes during canning. Therefore, the chemical composition was studied to investigate how the chemical properties influenced the structural and mechanical properties of the beans.

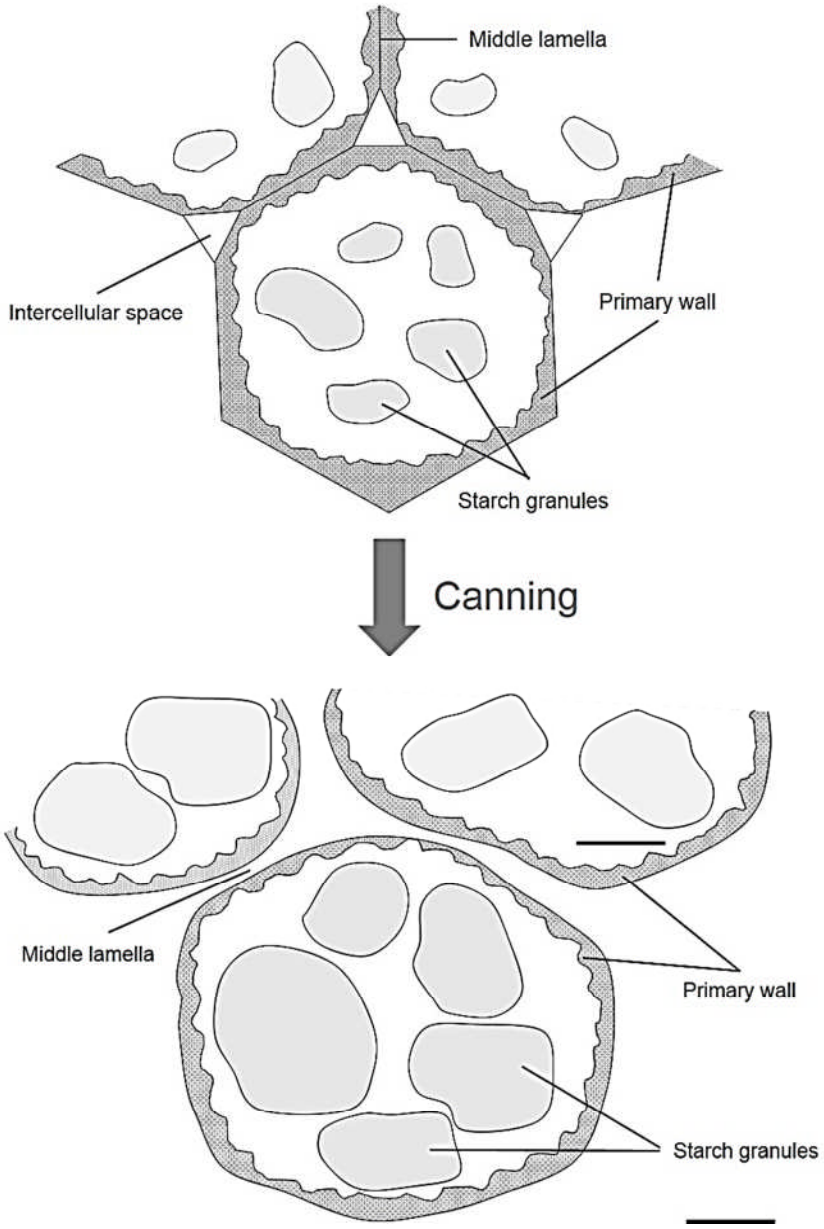

Figure 8.2 Diagram of the microstructure of the bean cells before and after canning.

Chemical composition

The dietary fibre content was investigated to study the cell wall material in the beans. Cultivar and region were found to have significant effect on dietary fibre content. A highly positive correlation was found between dietary fibre and beans firmness (r=0.9**), The higher amount of fibre may contribute to the thicker cell wall observed for the firmer beans, and firmer beans had lower pectin solubility (WSP:WIP ratio) compared to softer beans.

The dietary fibre (including WIP and WSP) were hydrolysed with 2M TFA at 120ºC for 1h to investigate the monosaccharide composition of these extracts. Arabinose was found to be the predominant neutral monosaccharide hydrolysed from bean dietary fibre (approximately 61% of hydrolysed sugars). The xylose and mannose were the second most abundant (approximately 22%) and followed by galactose (approximately 17 %). Region and cultivar were found to have significant effect on the neutral sugar (especially arabinose and galactose rich pectin). Beans that had more neutral sugar were suggested to have longer side chain length or more branched side chains.

Besides the cell wall material, other carbohydrate components were also studied in this project. Canned baked beans contained 9.14 – 11.48g starch content in every 100g of beans (wet weight basis). Beans had higher fibre content was found to have lower amount of starch, it is possible that the cell wall enclosing the starch granules made the starch difficult to isolate and therefore result in higher fibre content. No significant differences for reducing sugar (raffinose and stachyose) content were found between different cultivars and regions.

Storage effect

Storage effect was found in this project, beans stored at room temperature in an open package for 6 month was found 4.8-6.8 times tougher than fresh beans. The soluble pectin decreased 30% - 60% in 3 months storage. For legumes, such as yellow pea, brown bean, lentils and peas, storage was found harden the texture of the legumes significantly (Shiga et al., 2004, Detoro, 1993, Iliadis, 2001, Stanley et al., 1990). They claimed high temperature and high humidity would harden the beans, which associated with loss of uronic acid in water-soluble polymers and the loss in the solubility of neutral polymer (Ara-rich polymers). 4°C was suggested to be the best storage condition since it prevent hard-to-cook phenomenon (Shiga et al., 2004). Therefore, for industry, it is important to understand the response of beans to storage conditions, the knowledge may lead to improved efficiency in the food chain, while maintaining food quality at low cost. However, due to the time limitation, different storage conditions were not studied in this project. And it is very interesting to investigate in further studies.

Conclusion

In conclusion, cell wall polysaccharides played very important role in determining the texture quality of canned baked beans both structurally and chemically. Structurally they significantly affect cell adhesion, chemically the cell wall neutral sugar in WIP (especially Ara-rich and Gal-rich polymers) was positively correlated with the firmness of the beans. Cultivar and region were found significant affect the mechanical properties (firmness of beans and viscosity of sauce) and chemical composition (carbohydrate composition including fibre, starch and water soluble pectin content) of navy beans. Processing was found to significantly influence the cell adhesion structurally and affect the solubility and depolymerisation of pectin chemically.

Future work

The determinants of the cultivar and region effect on the texture of beans.

This project found that cultivar and region had significant effect on the texture quality of canned beans. However, the reason and determinant that caused the differences are unknown. The differences on region may cause by the climate, soil, rainfall or humidity. Green house can be applied to simulate different growth region conditions to fix the influence factor, and investigate the determinant. Cultivar differences may cause by the differences in genotype. Gene functional analysis can be determined using PCR to specify any gene in the beans may related to the firmness of beans.

The other factors may affect the texture quality of beans

In this project, carbohydrate content of navy beans was focused to study cell wall polysaccharides and starch. Except dietary fibre and starch content, protein is also main component of navy beans. Legume seeds were found contain up to 25% of protein, and protein was found have a loss of 0.86% - 2.68% during thermal processing, and even higher amount of loss (1.33% - 4.58%) under high pressure (Rehman and Shah, 2005). Therefore, it is possible protein content may have significant effect on texture of canned beans, so that protein content is also very important to study.

In this project, cell wall pectin solubilisation was focused to study. Because the depolymerisation also happened during canning and intercellular pectin depolymerised during the cooking rather than solubilised (Walter and Taylor, 1991), therefore the depolymerisation of the pectin can be studied further and deeper. Size-exclusion chromatography (SEC) can be used to measure the pectin molecule size in order to study the depolymerisation of the pectin.

List of Abbreviations

Ara	arabinose
CL	chain length
CWM	cell wall monosaccharides
DP	degree of polymerizatio
ELISA	The enzyme-linked immunosorbent assay
FITC	fluorescein isothiocyanate
Fuc	fucose
Gal	galactose
GalUA	galacturonic acid
Glc	glucose
GlcA	glucuronic acid.
HG	Homogalacturonan
HPAEC-PAD	High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
Man	mannose
PBS	phosphate buffered saline
PME	pectin methylesterase
RGI	rhamnogalacturonan I
RGII	rhamnogalacturonan II
Rha	rhamnose
WIP	water insoluble polysaccharides
WSP	water soluble polysaccharides

Xyl	xylose

Acknowledgements

First and foremost, thank God for all the experience in my life and all the blessings. Thank Him for giving me wisdom, being my strength and guide throughout my final year of my PhD.

I would like to express my special appreciation and deepest thanks to my primary supervisor Dr. Caroline Orfila. Caroline have been showing her tremendous patience and kindness during the four years. I am truly thankful for her encouragement and wise counsel throughout my PhD. From her I learned a lot of knowledge and the vital skill of critical thinking. Without her I would not have my PhD finished and I cannot think of a better supervisor to have.

I would also like to thank my second supervisor Dr. Peter Ho. Thank him for taking time to help me sort out statistic problems, and being my mentor guiding me through every steps of my work. Being kind and patience always gave me great encouragement for my study.

Thanks to my industrial supervisor Mr. Barry McConnie and Mr. Graham Tregonning from Heinz. Thanks to the generous sponsorship from Heinz, and Barry and Graham's kindness and supports during every meeting in the past four years.

Thanks to Prof. Paul Knox from school of Biology, whom generously provided us all the antibodies we need throughout all my PhD work. Also gave wise suggestions and advice for my work.

I would like to thank the following professors for their help at various stages of my research project, Prof. Malcolm Povey, Prof. Brent Murray, Dr. Melvin Holmes and Dr. Jianshe Chen. I would also would like to say thanks to my colleagues in Dr. Caroline Orfila's research group, for their kindness and being supportive. Especially thanks to my colleague Sarah Al Hamli, who gave me great help and support in my first 2 years of PhD.

I am extremely grateful to my parents for their unconditional love, caring and sacrifices for educating me and support for my PhD. I know I always have my families to rely on whenever I need them, I love them so much and I can never make it this far without them. I wish to thank my dearest friend Mr. Chee Horng Chang

and Mrs. Ping Ping Sii, they helped me through my toughest time in UK and supported me by all means. I also would like to say thanks to all my friends for their contributed support and friendship.

References

ABU-GHANNAM, N. 1998. Modelling textural changes during the hydration process of red beans. *Journal of Food Engineering,* 38**,** 341-352.

ALBERSHEIM, P., DARVILL A., ROBERTS K., SEDEROFF R. & A., S. 2011. *Plant cell walls : from chemistry to biology* New York, Garland Science. .

ALBERSHEIM, P., DARVILL, A. G., ONEILL, M. A., SCHOLS, H. A. & VORAGEN, A. G. J. 1996. An hypothesis: The same six polysaccharides are components of the primary cell walls of all higher plants. *Pectins and Pectinases,* 14**,** 47-55.

ALDWAIRJI, M. A., CHU, J., BURLEYB, V. J. & ORFILA, C. 2014. Analysis of dietary fibre of boiled and canned legumes commonly consumed in the United Kingdom. *Journal of Food Composition and Analysis,* 36**,** 111-116.

ANDERSON, J. W., STORY, L., SIELING, B., CHEN, W. J. L., PETRO, M. S. & STORY, J. 1984. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition,* 40**,** 1146-1155.

ANDERSSON, A., GEKAS, V., LIND, I., OLIVEIRA, F., ÖSTE, R. & AGUILFRA, J. M. 1994. Effect of preheating on potato texture. *Critical Reviews in Food Science and Nutrition,* 34**,** 229-251.

ANTHON, G. E. & BARRETT, D. M. 2006. Characterization of the temperature activation of pectin methylesterase in green beans and tomatoes. *Journal of Agricultural and Food Chemistry,* 54**,** 204-211.

ANZALDUA-MORALES, A. & BRENNAN, J. G. 1982. Relationship between the physical properties of dried beans and their textural characteristics after processing. *Journal of Texture Studies,* 13**,** 229-240.

AOAC 1995. Association of official analytical chemists: Offical method of Analysis. 16th edition. . Arlington. VA.

BALASUBRAMANIAN, P., SLINKARD, A., TYLER, R. & VANDENBERG, A. 2000. A modified laboratory canning protocol for quality evaluation of dry bean (Phaseolus vulgaris L.). *Journal of the Science of Food and Agriculture,* 80**,** 732-738.

BARTOLOME, L. G. & HOFF, J. E. 1972. Firming of potatoes. Biochemical effects of preheating. *Journal of Agricultural and Food Chemistry,* 20**,** 266-270.

BAY, A. P. M., BOURNE, M. C. & TAYLOR, A. G. 1996. Effect of moisture content on compressive strength of whole snap bean (Phaseolus vulgaris L) seeds and separated cotyledons. *International Journal of Food Science and Technology,* 31**,** 327-331.

BAZZANO, L. A., HE, J., OGDEN, L. G., LORIA, C., VUPPUTURI, S., MYERS, L. & WHELTON, P. K. 2001. Legume consumption and risk of coronary heart disease in US men and women. *Archives of Internal Medicine,* 161**,** 2573-2578.

BAZZANO, L. A., THOMPSON, A. M., TEES, M. T., NGUYEN, C. H. & WINHAM, D. M. 2011. Non-soy legume consumption lowers cholesterol levels: A meta-analysis of randomized controlled trials. *Nutrition Metabolism and Cardiovascular Diseases,* 21**,** 94-103.

BERG, T., SINGH, J., HARDACRE, A. & BOLAND, M. J. 2012. The role of cotyledon cell structure during in vitro digestion of starch in navy beans. *Carbohydrate Polymers,* 87**,** 1678-1688.

BILIADERIS, C. G., GRANT, D. R. & VOSE, J. R. 1981. Structural characterization of legume starches.I. Studies on amylose, amylopectin and beta-limit dextrins. *Cereal Chemistry Journal,* 58**,** 496-502.

BOURNE, M. C. 2002. *Food texture and viscosity : concept and measurement,* San Diego, Academic Press.

BRETT, C. & WALDRON, K. 1996. *Physiology and biochemistry of plant cell walls (2nd ed.),* London, Chapman and Hall.

BRITISH NURTITION FOUNDATION, B. 1990. Complex carbohydrates in foods: The report of the British Nutrition Foundation's task force. . Chapman and Hall for British Nurtition Foundation.

BUSH, M. S., MARRY, M., HUXHAM, I. M., JARVIS, M. C. & MCCANN, M. C. 2001. Developmental regulation of pectic epitopes during potato tuberisation. *Planta,* 213**,** 869-880.

CAFFALL, K. H. & MOHNEN, D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research,* 344**,** 1879-900.

CHO, S. S. 1999. *Complex carbohydrates in foods (food science and technology)*, CRC Press

CHUNG, H. J., LIU, Q. & HOOVER, R. 2010. Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches. *Food Research International,* 43**,** 501-508.

CLARK, W. D., STERN, K. R., VODOPICH, D. & MOORE, R. 1995. *Botany*, William C Brown Pub.

CLAUSEN, M. H., WILLATS, W. G. T. & KNOX, J. P. 2003. Synthetic methyl hexagalacturonate hapten inhibitors of antihomogalacturonan monoclonal antibodies LM7, JIM5 and JIM7. *Carbohydrate Research,* 338**,** 1797-1800.

COSTA, G. E. D., QUEIROZ-MONICI, K. D. S., REIS, S. M. P. M. & DE OLIVEIRA, A. C. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chemistry,* 94**,** 327-330.

COSTA, N. M. B., F, A., WALKER & LOW, A. G. 1993. The effect of graded inclusion of baked beans (Phaseolus vulgaris) on plasma and liver lipids in hypercholesterolaemic pigs given a Western-type diet. *British Journal of Nutrition,* 70**,** 515-524.

DAUSSANT, J., MOSSE, J. & VAUGHAN, J. 1983. *Seed proteins*, Acdemic press.

DAVIS, D. R., TWOGOOD, M. L. & BLACK, K. R. 1980. Effect of blanch treatment on quality attributes of canned dry pinto and small and large lima beans. *Journal of Food Science,* 45**,** 817-820.

DE-MEJIA, E. G., CASTANO-TOSTADO, E. & LOARCA-PINA, G. 1999. Antimutagenic effects of natural phenolic compounds in beans.

Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 441**,** 1-9.

DE LANGE, A. & LABUSCHAGNE, M. 2001. Multivariate assessment of canning quality, chemical characteristics and yield of small white canning beans (Phaseolus vulgaris L) in South Africa. *Journal of the Science of Food and Agriculture,* 81**,** 30-35.

DETORO, A. 1993. Influence of storage-temperature and moisture-content on cooking time of yellow peas and brown beans. *Swedish Journal of Agricultural Research,* 23**,** 191-197.

DRUMM, T. D., GRAY, J. I. & HOSFIELD, G. L. 1990. Variability in the saccharide, protein, phenolic-acid and saponin contents of 4 market classes of edible dry beans. *Journal of the Science of Food and Agriculture,* 51**,** 285-297.

ECKARDT, N. A. 2008. Role of xyloglucan in primary cell walls. *Plant Cell,* 20**,** 1421-2.

FRY, S. C. 1988. *The growing plant cell wall: chemical and metabolic analysis,* United Kingdom, British Library Cataloguing in Publication Data.

GEIL, P. B. & ANDERSON, J. W. 1994. Nutrition and health implications of dry beans - a review. *Journal of the American College of Nutrition,* 13**,** 549-558.

GIBNEY, M. J. 1982. Hypocholesterlesterolaemic effect of soya-bean proteins. *Proceedings of Nutrition Society* 41**,** 19-26.

HAHN, D. M., JONES, F. T., VAN AKHA, I. & ROCKLAND, L. B. 1977. LIght and scanning electron microscope studies on dry beans: intracellular gelatinization of starch in cotyledons of large lima beans (phaseolus lunatus). *Journal of Food Science,* 42**,** 1208-1212.

HARVARD UNIVERSITY 2004. Fiber Content of Foods in Common Portions.

HELLENDO, E. 1969. Intestinal effects following ingestion of beans. *Food Technology,* 23**,** 795-&.

HENDERSON, L., Y, G. & SWAN, G. 2002. Adults aged 19 to 64 years, types of quantities of foods consumed. *National diet and nutrition survey,* 4.

HENSHALL, A. 1999. High performance anion exchange chromatography with pulsed amperometric detection (HPAE- PAD). *Complex Carbohydrates in Foods.* CRC Press.

HOOVER, R. & RATNAYAKE, W. S. 2002. Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry,* 78**,** 489-498.

HOOVER, R. & SOSULSKI, F. 1985. Studies on the functional-characteristics and digestibility of starches from phaseolus-vulgaris biotypes. *Starke,* 37**,** 181-191.

HOSFIELD, G. L., UEBERSAX, M. A. & ISLEIB, T. G. 1984. Seasonal and genotypic effects on yield and physico-chemical seed characteristics related to food quality in dry, edible beans. *Journal of the American Society for Horticultural Science,* 109**,** 182-189.

ILIADIS, C. 2001. Effects of harvesting procedure, storage time and climatic conditions on cooking time of lentils (Lens culinaris Medikus). *Journal of the Science of Food and Agriculture,* 81**,** 590-593.

ILKER, R. & SZCZESNIAK, A. S. 1990. Structural and chemical bases for texture of plant foodstuffs. *Journal of Texture Studies,* 21**,** 1-36.

ISHII, T. & MATSUNAGA, T. 2001. Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan. *Phytochemistry,* 57**,** 969-974.

JARVIS, M. C. 1998. Intercellular separation forces generated by intracellular pressure. *Plant Cell and Environment,* 21**,** 1307-1310.

JARVIS, M. C., BRIGGS, S. P. H. & KNOX, J. P. 2003. Intercellular adhesion and cell separation in plants. *Plant Cell and Environment,* 26**,** 977-989.

JOKANOVIĆ, M. R., DŽINIĆ, N. R., CVETKOVIĆ, B. R., GRUJIĆ, S. & ODŽAKOVIĆ, B. 2012. Changes of physical properties of coffee beans during roasting *APTEFF,* 43**,** 342.

JUNEK, J. J., SISTRUNK, W. A. & NEELY, M. B. 1980. Influence of processing methodology on quality attributes of canned dry beans. *Journal of Food Science,* 45**,** 821-824.

KAACK, K. 1994. Blanching of green bean (phaseolus-vulgaris). *Plant Foods for Human Nutrition,* 46**,** 353-360.

KERELIUK, G. R. & KOZUB, G. C. 1995. Chemical composition of small white (navy) beans. *Food Science and Technology,* 28**,** 272-278.

KINGMAN, S. M. 1991. The influence of legume seeds on human plasma lipid concentrations. *Nutrition Research Reviews,* 4**,** 97-123.

KNOX, J. P. 1997. The use of antibodies to study the architecture and developmental regulation of plant cell walls. *International Review of Cytology - a Survey of Cell Biology,* 171**,** 79-120.

KNOX, J. P., LINSTEAD, P. J., KING, J., COOPER, C. & ROBERTS, K. 1990. Pectin esterification is spatially regulated both within cell-walls and between developing-tissues of root apices. *Planta,* 181**,** 512-521.

KRAMER, A. & AND SZCZESNIAK, A. S. 1973. *Texture measurements of foods*, D. Reidel Publishing Company.

KRAMER, A. & SZCZESNIAK, A. S. 1973. *Texture measurements of food; psychophysical fundamentals, sensory, mechanical, and chemical procedures, and their interrelationships,* Dordrecht, Boston,, D. Reidel Pub. Co.

LIN, Z. & SCHYVENS, E. 1995. Influence of blanching treatments on the texture and color of some processed vegetables and fruits. *Journal of Food Processing and Preservation,* 19**,** 451-465.

LIU, K. 1995. Cellular, biological, and physicochemical basis for the hard-to-cook defect in legume seeds. *Critical Reviews in Food Science and Nutrition,* 35**,** 263-298.

LIU, K. S. & MCWATTERS, K. H. 1994. Effects of storage, soaking and cooking method on cookability of cowpeas. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie,* 27**,** 295-295.

LOWE, R. B. 2012. *Changes in carbohydrates of navy beans during hydration and subsequent thermal processing.* University of Tennessee, Knoxville.

LU, W. & CHANG, K. C. 1996. Correlations between chemical composition and canning quality attributes of navy bean (Phaseolus vulgaris L). *Cereal Chemistry,* 73**,** 785-787.

LUH, B. S., WANG, C. & DAOUD, H. N. 1975. Several factors affecting color, texture and drained weight of canned dry lima beans. *Journal of Food Science,* 40**,** 557-561.

LUHOVYY, B. L., MOLLARD, R. C., PANAHI, S., NUNEZ, M. F., CHO, F. & ANDERSON, H. 2015. Canned Navy Bean Consumption Reduces Metabolic Risk Factors Associated with Obesity. *Canadian Journal of Dietetic Practice and Research,* 76**,** 33-37.

LUTHRIA, D. L. & PASTOR-CORRALES, M. A. 2006. Phenolic acids content of fifteen dry edible bean (Phaseolus vulgaris L.) varieties. *Journal of Food Composition and Analysis,* 19**,** 205-211.

MANFIELD, I. W., BERNAL, A. J., MOLLER, I., MCCARTNEY, I., RIESS, N. P., KNOX, J. P. & WILLATS, W. G. T. 2005. Re-engineering of the PAM1 phage display monoclonal antibody to produce a soluble, versatile anti-homogalacturonan scFv. *Plant Science,* 169**,** 1090-1095.

MARCUS, S. E., BLAKE, A. W., BENIANS, T. A. S., LEE, K. J. D., POYSER, C., DONALDSON, L., LEROUX, O., ROGOWSKI, A., PETERSEN, H. L., BORASTON, A., GILBERT, H. J., WILLATS, W. G. T. & KNOX, J. P. 2010. Restricted access of proteins to mannan polysaccharides in intact plant cell walls. *Plant Journal,* 64**,** 191-203.

MARCUS, S. E., VERHERTBRUGGEN, Y., HERVE, C., ORDAZ-ORTIZ, J. J., FARKAS, V., PEDERSEN, H. L., WILLATS, W. G. & KNOX, J. P. 2008. Pectic homogalacturonan masks abundant sets of xyloglucan epitopes in plant cell walls. *BMC Plant Biology,* 8**,** 60.

MCCAN, M. C., WELLS, B. & ROBERTS, K. 1990. Direct visualization of cross-links in the primary plant cell wall. *Journal of cell science,* 96**,** 323-334.

MCCARTNEY, L. & KNOX, J. P. 2002. Regulation of pectic polysaccharide domains in relation to cell development and cell properties in the pea testa. *Journal of Experimental Botany,* 53**,** 707-713.

MCCARTNEY, L., ORMEROD, A. P., GIDLEY, M. J. & KNOX, J. P. 2000. Temporal and spatial regulation of pectic (1 -> 4)-beta-D-galactan in cell walls of developing pea cotyledons: implications for mechanical properties. *Plant Journal,* 22**,** 105-113.

MCNEIL, M., DARVILL, A. G., FRY, S. C. & ALBERSHEIM, P. 1984. Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry,* 53**,** 625-63.

MOHNEN, D. 2008. Pectin structure and biosynthesis. *Current Opinion in Plant Biology,* 11**,** 266-277.

MOHSENIN, N. N., COOPER, H. E. & TUKEY, L. D. 1963. Engineering approach to evaluating textural factors in fruits and vegetables. *Transactions of the ASAE. American Society of Agricultural Engineers (T ASABE),* 6**,** 85-88.

MOLLARD, R. C., LUHOVYY, B. L., PANAHI, S., NUNEZ, M., HANLEY, A. & ANDERSON, G. H. 2012. Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults. *British Journal of Nutrition,* 108**,** S111-S122.

NORDSTROM, C. L. & SISTRUNK, W. A. 1977. Effect of type of bean, soak time, canning media and storage time on quality attributes and nutritional value of canned dry beans. *Journal of Food Science,* 42**,** 795-798.

NORDSTROM, C. L. & SISTRUNK, W. A. 1979. Effect of type of bean, moisture level, blanch treatment and storage time on quality attributes and nutrient content of canned dry beans. *Journal of Food Science,* 44**,** 392-&.

O'NEILL, M. A. & YORK, W. S. 2003. The composition and structure of plant primary cell walls. *In:* ROSE, K.C. (ed.) *The plant cell wall.* Blackwell Publishing CRC Press.

OBRO, J., HARHOLT, J., SCHELLER, H. V. & ORFILA, C. 2004. Rhamnogalacturonan I in Solanum tuberosum tubers contains complex arabinogalactan structures. *Phytochemistry,* 65**,** 1429-38.

ORDAZ-ORTIZ, J. J., MARCUS, S. E. & KNOX, J. P. 2009. Cell wall microstructure analysis implicates hemicellulose polysaccharides in

cell adhesion in tomato fruit pericarp parenchyma. *Molecular Plant,* 2**,** 910-921.

OREGON STATE UNIVERSITY 2012. Composition of Sugars, Oregon State University Oregon State University.

ORFILA, C. 2001. *Structure and function of the plant cell wall in relation to cell adhesion in ripening tomato fruit.* PhD, University of Leeds.

ORFILA, C., HUISMAN, M. M. H., WILLATS, W. G. T., VAN ALEBEEK, G. J. W. M., SCHOLS, H. A., SEYMOUR, G. B. & KNOX, J. P. 2002. Altered cell wall disassembly during ripening of Cnr tomato fruit: implications for cell adhesion and fruit softening. *Planta,* 215**,** 440-447.

ORFILA, C. & KNOX, J. P. 2000. Spatial regulation of pectic polysaccharides in relation to pit fields in cell walls of tomato fruit pericarp. *Plant Physiology,* 122**,** 775-81.

ORFILA, C., SEYMOUR, G. B., WILLATS, W. G., HUXHAM, I. M., JARVIS, M. C., DOVER, C. J., THOMPSON, A. J. & KNOX, J. P. 2001. Altered middle lamella homogalacturonan and disrupted deposition of (1-->5)-alpha-L-arabinan in the pericarp of Cnr, a ripening mutant of tomato. *Plant Physiology,* 126**,** 210-21.

PHATAK, L., CHANG, K. C. & BROWN, G. 1988. Isolation and characterization of pectin in sugar-beet pulp. *Journal of Food Science,* 53**,** 830-833.

PROCTOR, J. P. & WATTS, B. M. 1987. Effect of cultivar, growing location, moisture and phytate content on the cooking times of freshly harvested navy beans. *Canadian Journal of Plant Science,* 67**,** 923-926.

QUENZER, N. M., HUFFMAN, V. L. & BURNS, E. E. 1978. Some factors affecting pinto bean quality. *Journal of Food Science,* 43**,** 1059-1061.

RAVEN, P. H., EVERT, R. F. & EICHHORN, S. E. 2012. *Biology of plants* W. H. Freeman and Company Publishers.

REBELLO, C. J., GREENWAY, F. L. & FINLEY, J. W. 2014. A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. *Obesity Reviews,* 15**,** 392-407.

REHMAN, Z. U. & SHAH, W. H. 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry,* 91**,** 327-331.

RENARD, C. M. G. C. & JARVIS, M. C. 1999. A cross-polarization, magic-angle-spinning,13c-nuclear-magnetic-resonance study of polysaccharides in sugar beet cell walls. *Plant Physiology,* 119**,** 1315-1322.

REVILLA, I. & VIVAR-QUINTANA, A. M. 2008. Effect of canning process on texture of Faba beans (Vicia Faba). *Food Chemistry,* 106**,** 310-314.

ROCKLAND, L. B. & JONES, F. T. 1974. Scanning electron-microscope studies on dry beans - effects of cooking on cellular structure of cotyledons in rehydrated large lima beans. *Journal of Food Science,* 39**,** 342-346.

ROSENTHAL, A. J. 1999. *Food texture : measurement and perception,* Gaithersburg, Md., Aspen Publishers.

SAJJAANANTAKUL, T., VAN BUREN, J. P. & DOWNING, D. L. 1989. Effect of methyl ester content on heat degradation of chelator-soluble carrot pectin. *Journal of Food Science,* 54**,** 1272-1277.

SANCHEZ-MATA, M. C., PENUELA-TERUEL, M. J., CAMARA-HURTADO, M., DIEZ-MARQUES, C. & TORIJA-ISASA, M. E. 1998. Determination of mono-, di-, and oligosaccharides in legumes by high-performance liquid chromatography using an amino-bonded silica column. *Journal of Agricultural and Food Chemistry,* 46**,** 3648-3652.

SAT, I. G. & KELES, F. 2002. The effect of soaking and cooking on the oligosaccharide content of seker a dry bean variety (p. vulgaris, l) grown in Turkey. *Pakistan Journal of Nutrition,* 1**,** 206-208.

SCHELLER, H. V., JENSEN, J. K., SORENSEN, S. O., HARHOLT, J. & GESHI, N. 2007. Biosynthesis of pectin. *Physiologia Plantarum,* 129**,** 283-295.

SCOTT, R. W. 1979. Colorimetric determination of hexuronic acids in plant materials. *Analytical Chemistry,* 51**,** 936-941.

SEFADEDEH, S., STANLEY, D. W. & VOISEY, P. W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (vigna-unguiculata). *Journal of Food Science,* 43**,** 1832-1838.

SELVENDRAN, R. R. 1984. The plant cell wall as a source of dietary fiber: chemistry and structure. *The American Journal of Clinical Nutrition,* 39**,** 320-37.

SHAMA, F. & SHERMAN, P. 1973. Identification of stimuli controlling the sensory evaluation of viscosity ii. oral methods. *Journal of Texture Studies,* 4**,** 111-118.

SHIGA, T. M. & LAJOLO, F. M. 2006. Cell wall polysaccharides of common beans (Phaseolus vulgaris L.) - Composition and structure. *Carbohydrate Polymers,* 63**,** 1-12.

SHIGA, T. M., LAJOLO, F. M. & FILISETTI, T. M. C. C. 2004. Changes in the cell wall polysaccharides during storage and hardening of beans. *Food Chemistry,* 84**,** 53-64.

SHRIVASTAVA, A. & GUPTA, V. B. 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists,* 2**,** 21-25.

SHUTLERA;, S. M., BIRCHERA;, G. M., TREDGERA;, J. A., MORGANA;, L. M., WALKERA;, A. F. & LOWA, A. G. 1989. The effect of daily baked bean (Phaseolus vulgaris) consumption on the plasma lipid levels of young, normo-cholesterolaemic men. *British Journal of Nutrition,* 61**,** 257-265.

SIRTORI, C. R., GATTI, E., MANTERO, CONTI, F., AGRADI, E., TREMOLI, E., SIRTORI, M., FRATERRIGO, L., TAVAZZI, L. & KRITCHEVSKY, D. 1979. Clinical experience with the soybean protein diet in the treatment of hypercholesterolemia. *The American Journal of Clinical Nutrition,* 32**,** 1645-1658.

SMALLWOOD, M., MARTIN, H. & KNOX, J. P. 1995. An epitope of rice threonine- and hydroxyproline-rich glycoprotein is common to cell wall and hydrophobic plasma-membrane glycoproteins. *Planta,* 196**,** 510-522.

SRISUMA, N., RUENGSAKULRACH, S., UEBERSAX, M. A., BENNINK, M. R. & HAMMERSCHMIDT, R. 1991. Cell-Wall Polysaccharides of Navy Beans (Phaseolus-Vulgaris). *Journal of Agricultural and Food Chemistry,* 39**,** 855-858.

STANLEY, D. W., MICHAELS, T. E., PLHAK, L. C. & CALDWELL, K. B. 1990. Storage-Induced Hardening in 20 Common Bean Cultivars. *Journal of Food Quality,* 13**,** 233-247.

STEGGERD, F. 1968. Gastrointestinal gas following food consumption. *Annals of the New York Academy of Sciences,* 150**,** 57-66.

STOLLE-SMITS, T., BEEKHUIZEN, J. G., KOK, M. T., PIJNENBURG, M., RECOURT, K., DERKSEN, J. & VORAGEN, A. G. 1999. Changes in cell wall polysaccharides of green bean pods during development. *Plant Physiology,* 121**,** 363-72.

STOLLE-SMITS, T., BEEKHUIZEN, J. G., RECOURT, K., VORAGEN, A. G. & VAN DIJK, C. 2000. Preheating effects on the textural strength of canned green beans. 1. Cell wall chemistry. *Journal of Agricultural and Food Chemistry,* 48**,** 5269-77.

STOLLE-SMITS, T., BEEKHUIZEN, J. G., VANDIJK, C., VORAGEN, A. G. J. & RECOURT, K. 1995. Cell-Wall dissolution during industrial processing of green beans (phaseolus-vulgaris l). *Journal of Agricultural and Food Chemistry,* 43**,** 2480-2486.

STOLLE-SMITS, T., DONKERS, J., VAN DIJK, C., DERKSEN, J. & SASSEN, M. M. A. 1998. An electron microscopy study on the texture of fresh, blanched and sterilized green bean pods (Phaseolus vulgaris L.). *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie,* 31**,** 237-244.

TANAKA, M., THANANUNKUL, D., LEE, T. C. & CHICHESTER, C. O. 1975. A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. *Journal of Food Science,* 40**,** 1087-1088.

TIEMAN, D. M., HARRIMAN, R. W., RAMAMOHAN, G. & HANDA, A. K. 1992. An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. *Plant Cell,* 4**,** 667-679.

TOPPING, D. L., TRIMBLE, R. P., ILLMAN, R. J., POTTER, J. D. & OAKENFULL, D. G. 1984. Prevention of dietary hypercholesterolemia in the rat by soy flour high and low in saponins. *Nutrition Reports International* 22**,** 513-519.

UNIVERSITY OF WISCONSIN 2007. Starch digestibility; degree of starch access. University of Wisconsin Soil Testing Laboratories.

VALEN, L. V. 1970. Biometry. The Principles and Practice of Statistics in Biological Research. Robert R. Sokal and F. James Rohlf. xxii + 778 pp., illus. $15. Biology Series. Statistical Tables. F. James Rohlf and Robert R. Sokal. xiV + 258 pp. Cloth, $7.50; paper, $2.75. Freeman, San Francisco, 1969. *Science,* 167**,** 165.

VALENCIA, C., SANCHEZ, M. C., CIRUELOS, A., LATORRE, A., FRANCO, J. M. & GALLEGOS, C. 2002. Linear viscoelasticity of tomato sauce products: influence of previous tomato paste processing. *European Food Research and Technology,* 214**,** 394-399.

VAN DER MERWE, D., OSTHOFF, G. & PRETORIUS, A. J. 2006. Comparison of the canning quality of small white beans (Phaseolus vulgaris L.) canned in tomato sauce by a small-scale and an industrial method. *Journal of the Science of Food and Agriculture,* 86**,** 1046-1056.

VANDENBOSCH, K. A., BRADLEY, D. J., KNOX, J. P., PEROTTO, S., BUTCHER, G. W. & BREWIN, N. J. 1989. Common components of the infection thread matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. *The EMBO Journal* 8**,** 335-41.

VARELA, R. O. & ALBORNOZ, P. L. 2013. Morpho-anatomy, imbibition, viability and germination of the seed of Anadenanthera colubrina var. cebil (Fabaceae). *Revista de biologia tropical,* 61**,** 1109-18.

VERHERTBRUGGEN, Y., MARCUS, S. E., HAEGER, A., ORDAZ-ORTIZ, J. J. & KNOX, J. P. 2009. An extended set of monoclonal antibodies to pectic homogalacturonan. *Carbohydrate Research,* 344**,** 1858-1862.

VOISEY, P. W. 1971. Ottawa Texture Measuring System. *Canadian Institute of Food Science and Technology Journal,* 4**,** 91-103.

VOISEY, P. W. & LARMOND, E. 1971. Texture of baked beans - a comparison of several methods of measurement. *Journal of Texture Studies,* 2**,** 96-109.

WALDRON, K. W., SMITH, A. C., PARR, A. J., NG, A. & PARKER, M. L. 1997. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends in Food Science & Technology,* 8**,** 213-221.

WALTER, R. H. & TAYLOR, S. 1991. *The Chemistry and Technology of Pectin*, Food Science and Technology.

WALTERS, K. J., HOSFIELD, G. L., UEBERSAX, M. A. & KELLY, J. D. 1997. Navy bean canning quality: Correlations, heritability estimates, and randomly amplified polymorphic DNA markers associated with component traits. *Journal of the American Society for Horticultural Science,* 122**,** 338-343.

WANG, C. C. R. & CHANG, S. K. C. 1988. Effect of selected canning methods on trypsin-inhibitor activity, sterilization value, and firmness of canned navy beans. *Journal of Agricultural and Food Chemistry,* 36**,** 1015-1018.

WANG, C. R., CHANG, K. C. & GRAFTON, K. 1988. Canning quality evaluation of pinto and navy beans. *Journal of Food Science,* 53**,** 772-776.

WARSAME, A. O. A. K., P.M. 2014. Canning quality of new drought-tolerant dry bean (Phaseolus vulgaris L.) lines. *American Journal of Food Technology,* 9**,** 311-317.

WIIRSCH, P., VEDOVO, S. D. & KOELLREUTTER, B. 1986. Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *The American Journal of Clinical Nutrition,* 43**,** 25-29.

WILLATS, W. G., LIMBERG, G., BUCHHOLT, H. C., VAN ALEBEEK, G. J., BENEN, J., CHRISTENSEN, T. M. I. E., VISSER, J., VORAGEN, A., MIKKELSEN, J. D. & KNOX, J. P. 2000. Analysis of pectin structure part 2 - Analysis of pectic epitopes recognised by hybridoma and phage display monoclonal antibodies using defined oligosaccharides,

polysaccharides, and enzymatic degradation. *Carbohydrate Research,* 327**,** 309-320.

WILLATS, W. G., MARCUS, S. E. & KNOX, J. P. 1998. Generation of monoclonal antibody specific to (1-->5)-alpha-L-arabinan. *Carbohydrate Research,* 308**,** 149-52.

WILLATS, W. G., MCCARTNEY, L., MACKIE, W. & KNOX, J. P. 2001a. Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology,* 47**,** 9-27.

WILLATS, W. G., ORFILA, C., LIMBERG, G., BUCHHOLT, H. C., VAN ALEBEEK, G. J., VORAGEN, A. G., MARCUS, S. E., CHRISTENSEN, T. M., MIKKELSEN, J. D., MURRAY, B. S. & KNOX, J. P. 2001b. Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls. Implications for pectin methyl esterase action, matrix properties, and cell adhesion. *Journal of Biological Chemistry,* 276**,** 19404-13.

WINHAM, D. M. & HUTCHINS, A. M. 2007. Baked bean consumption reduces serum cholesterol in hypercholesterolemic adults. *Nutrition research (New York),* 27**,** 380-386.

ZHAO, B. & CHANG, K. C. 2008. Evaluation of effects of soaking and precooking conditions on the quality of precooked dehydrated pea, lentil and chickpea products. *Journal of Food Processing and Preservation,* 32**,** 517-532.