School of Public Health

Characterisation of physicochemical properties of different oat cultivars used in noodle processing: effects on quality and β-glucan

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

SABORI MITRA

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Abstract

Oats grains have been highlighted for their positive contribution to human nutrition over many years. In addition to their favourable macronutrient profile, consumption of oats has the potential to lower risk factors for chronic diseases, mainly due to its β-glucan content. The total production of oats in Australia is increasing with 1096 kt produced in the 2014/2015 season. Australian milling oats are known for their superior nutritional quality, bright colour, taste and aroma. Thus, Australian oats have great potential to be incorporated in different food products. Oats have been mostly consumed as rolled oats in porridges, muesli and snack bars. However, in recent times whole oats are being incorporated in different food products due to increasing demand from consumers for healthier food products. One food product which could be used to incorporate oats is noodles. The popularity, simplicity in processing, low cost, fast and simple cooking methods of noodles make them ideal as a base to incorporate oats. In spite of oats' health benefits the major challenge of incorporation of oats into wheat based products such as noodles is that oats lack viscoelastic properties and the incorporation level required for health benefits affects the product quality and consumer acceptability. Hence it is important to identify ways that oats can be incorporated into new products which consumers will find attractive. Although, some additive or processing aids such as gluten and transglutaminase, have been reported to improve oat-wheat noodle quality. However, there is lack of information on effect of oat cultivars, growing seasons and the impact of processing on oat-wheat noodles, in terms of their sensory properties and factors related to nutritional quality such as β-glucan content and its susceptibility to breakdown due to processing.

Therefore, the main aim of this thesis was to characterise the physicochemical properties of different oat cultivars used in noodle processing and their effects on noodle quality and β-glucan content. Six oat cultivars from Western Australia (W.A.) (Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams) grown over two growing seasons (2011 and 2012) at the same location (Katanning) were selected for this research. The quality and isolated β-glucan properties of oat flour and 30% oat-wheat noodle (30% OW noodles) were evaluated and quality parameters were compared to standard wheat (udon) flour and noodles.

Physicochemical variations amongst the oat cultivars (particularly β-glucan content), were assessed to determine their suitability for incorporation into white salted noodle at a level of 30% of the flour component. The overall mean, for the six oat cultivars grown in 2012, was significantly higher ($P<0.05$) for protein content, lipid content and had a greater volume of finer particle size (<100 μm) flour, in comparison to the 2011 season samples. Furthermore the overall mean for the 2012 oat samples was lower for ash content, starch damage and had smaller volume of coarser particles (>100 μm), in comparison to the overall mean values for oat cultivars grown in 2011. The growing season (year) by cultivar interaction was significant ($P<0.05$) for ash content, protein content, β-glucan content, starch damage and particle size. Oat cultivar, Mitika, had highest peak viscosity for 100% oat flour and 30% oat-wheat flour blend; which may be due to its lower percent amylose, higher protein content and greater volume of finer particles.

The quality attributes of colour, colour stability, firmness and cooking solid loss of the 30% OW noodles made using 2012 oat flours were superior to the 30% OW noodles made using 2011 oat flours. The effect of growing season had greater impact on noodle firmness than the genetic effect of cultivars. The effect of genotype show that among the six oat cultivars, Williams had the highest β-glucan content (average 5.9%), however, it produced noodles with poor cooking and eating quality. Mitika, on the other hand, had a moderately high amount of β-glucan (average 5.2%), was found to be easier to handle during processing, and produced noodles with the superior brightness and colour stability in comparison to other oat cultivars evaluated.

The Australian milling oats studied varied in β-glucan content by one percent or less, but the β-glucan viscosity of the different cultivars varied more widely. Seasonal variation was evident with oat cultivars grown in 2012, having higher β-glucan viscosity than the same oat cultivar grown in 2011; for both oat flour and 30% OW noodle samples. Williams and Mitika were identified as cultivars which had high β-glucan content and the highest extracted β-glucan viscosity, pre and post processing into 30% OW noodles, for both years. Furthermore, β-glucan molecular weight of Williams for 2012 and Kojonup, for both years, was least affected by processing, with minimal change to molecular weight pre and post processing into 30% OW noodles.

Therefore, Williams and Mitika can be identified as having superior β-glucan content and properties with the potential for greater delivery of health benefits, as the maintenance of physicochemical properties is important in maintaining bioactivity of β-glucan. In summary, characterising Australian oat cultivars for differences in β-glucan viscosity, solubility, molecular weight and resistance to processing change is important in determining their suitability for incorporation into food products for providing health benefits. This research gave insight into the quality differences between oat cultivars, the effect of season on oat cultivar quality and the understanding of oat cultivar suitability for producing oat-wheat noodles with superior sensory, nutritional and health properties.

In future the potential of the selected oat cultivars to produce other products should be investigated. Different products for example bread or biscuits, have different processing steps and quality traits. Varied processing conditions may affect β-glucan properties differently, including development of viscosity. Also, different cultivars might be suitable for different oat incorporated products depending upon the product quality traits.

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- Mitra, S. 2014. Quality evaluation of oat flour and oat incorporated white salted noodles. Oat technical workshop (Australia-China Agricultural Cooperation Agreement Programme).AEGIC Australian Export Grains Innovation Centre. Perth, WA, Australia. September 15th-19th.

Conference presentations:

- Mitra, S., Cato, L., James, A.P., and Solah, V.A. and Fenton, H. 2013. Variability in physicochemical properties and their interaction in Western Australian oat cultivars. AGSA, Australian Cereal Chemistry Conference, Perth, Western Australia. August 25th- 28th. (Poster)

- Mitra, S., Cato, L., James, A.P., and Solah, V.A. and Fenton, H. 2014. Quality evaluation of oat incorporated white salted noodles. AGSA, Australian Cereal Chemistry Conference, Brisbane, Queensland. August 24th-27th. (Poster)

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Publications

- Mitra, S., Cato, L., James, A.P., and Solah, V.A. 2012. Evaluation of white salted noodles enriched with oat flour. Cereal Chem. 89(2): 117-125. Impact Factor 1.568.

- Mitra, S., Cato, L., James, A.P., and Solah, V.A., Fenton, H. 2015. The impact of oat quality on white salted noodles containing oat flour (submitted to Cereal Chemistry journal).

- Mitra, S., Lahnstein, J., Cato, L., James, A.P., and Solah, V.A., Fenton, H. 2015. Variability in (1,3);(1,4)-β-glucan viscosity and molecular weight change due to processing in Western Australian oat cultivars (to be submitted to Cereal Chemistry journal).

Chapter 1: Introduction

Oats, genus *Avena L.* (Poaceae) belong to the tribe *Aveneae* of the family Gramineae (Hareland and Manthey 2003; Zwer et al 2004). Oats are the sixth most produced cereal in the world following wheat, maize, rice, barley and sorghum (Ahmad et al 2014). Australia was among the top five countries in terms of oat production (Index Mundi (2013) with 1096 kt produced in the 2014 -2015 season (USDA 2015). Oat production in Australia is predicted to increase in 2015-2016 season. Western Australia is a leader in producing clean, safe and food grade oats, producing over 500 kt for human and animal consumption per year (AEGIC 2014a). In addition, Western Australian milling oats used for human consumption are produced in over 60% of the total area of Western Australia, sown to oats (DAFWA 2006).

Oats are more tolerant to wet weather, acidic soil and more resistant to foliar diseases and require less agro chemical and fertiliser inputs than other cereal crops (Hoffman 1995). Due to their resilience and ability to grow in wide range of soil types, oats generally survive in conditions where other cereals fail (Stevens et al 2004). Oats have been planted in China for more than 3000 years (Wang 2004a), but in Australia oats were mostly used as animal fodder until the 20th century when they were accepted as an important crop for human consumption. Oats have received increased interest in human nutrition research due to their dietary benefits such as containing nutritionally superior protein compared to other cereals (Brand and Van der Merwe 1996; Petkov et al 2001; Webster 2002; Sangwan et al 2014), being a good source of essential unsaturated fatty acids (Lásztity 1998; Webster 2002; Sangwan et al 2014), minerals, and antioxidants (Peterson et al 1975; Peterson 2001; Sangwan et al 2014). However, the main component of oats which is related to health benefits is the soluble dietary fibre β-glucan, present mainly in the cell wall of the aluerone layer of oat grain and partially in its endosperm (Hareland and Manthey 2003).

Health benefits of dietary fibre have been identified for decades, however, more recently the health benefits of soluble fibre such as (1→3, 1→4)-β-D-glucan (known as β-glucan) has been identified (Brennan and Cleary 2005). Cereal β-glucan has functional and nutritional properties due to its viscosity in aqueous systems and in the intestinal tracts (Dongowski et al 2005). The health benefits of β-glucan include: reduction in the risk of heart disease by reducing the elevated cholesterol in people

with high cholesterol; attenuation of post prandial glycaemic response or blood glucose level; and antihypertensive effect and improves satiety which helps in weight management (Ripsin et al 1992; Halfrisch et al 1995; Davy et al 2002; Pins et al 2002; Biorklund et al 2005; Dongowski et al 2005; Beck et al 2009). The European Union (EFSA 2009), U.S. Food and Drug Administration (2014) and FSANZ (2014) health claims for barley and oat β-glucans are related to the maintenance and reduction in the blood cholesterol with consumption of 3 g or more of β-glucan from oat and barley. The European Union health claims for barley and oat β-glucans require the consumption of 4 g β-glucans/30 g of available carbohydrate for the reduction of post-prandial glycaemia (EFSA 2011; Harland 2014). Due to these health claims and desired nutritional profile, oats are now incorporated into various cereal based food products such as breakfast cereals, granola bars, bread, muffins, noodles and cookies (Estévez et al 1995; Hellweg et al 1996; Zhang et al 1998; Lee and Inglett 2006; Flander et al 2007; Tosh et al 2008; Aigster et al 2011; Aydin and Gocmen 2011; Hüttner et al 2011; Ryan et al 2011; Mitra et al 2012). However, incorporation of oats tends to have an adverse effect on taste and texture of the products. Oats also lack the viscoelastic properties of wheat gluten which hinders its utilisation in wheat based products and consequently, most commercial oat products have a very low proportion of oats incorporated (Wang 2004b; Zhou et al 2011).

Researchers have shown that simplicity of noodles as an ideal base for incorporation of non-traditional ingredients, such as oats. However, the functionality of noodles incorporated with oats can be improved by changing the formulation and addition of additives such as transglutaminase, gluten and egg albumin (Wang 2004b; Wang et al 2011; Zhou et al 2011). In our laboratory we have identified Western Australian oat cultivars that are superior in the production of WSN in terms of processing ability and noodle quality (Mitra et al 2012). Previous studies have instead focused on the effects of altering the proportion of oat incorporation on noodle quality rather than varying cultivar (Aydin and Gocmen 2011; Majzoobi et al 2014).

Limited work has been done to investigate both the impact of environment (including year) and genetic variation of oats on the quality of end products (Lapveteläinen et al 2001; Rhymer et al 2005; Hüttner et al 2011; Mitra et al 2012). Seasonal conditions and cultivar variation of oats are known to affect oat composition and physicochemical properties such as pasting properties, β-glucan extractability, molecular weight and

fine structure (Doehlert et al 2001; Shewry et al 2008; Andersson and Börjesdotter 2011; Choi et al 2012; Doehlert and Simsek 2012; Stewart and McDougall 2014) and this would all have an impact on end product quality. It is therefore important to understand and identify which oat cultivars and growing environments are most suitable to produce oats with desirable properties for producing a particular oat based product.

Selection of high β-glucan containing oats could be one way to achieve the recommended amount of β-glucan, suggested by the health claims, but only if whole oats were consumed. However, the quantity of β-glucan consumed in diets cannot solely ensure the physiological efficiency. The effects of oat β glucan related to cholesterol lowering and postprandial glucose/lipid attenuation are attributed to the high viscosity of the polysaccharide within the lumen of the small intestine where it delays intestinal digestion, absorption, cholesterol absorption and reabsorption of bile acids (Webster 2002; Anttila et al 2004).

Factors that influence the β glucan solution viscosity are concentration, solubilisation and molecular weight (MW) of the polymer (Beer et al 1997a; Rimsten et al 2003; Ajithkumar et al 2005; Wolever et al 2010). These β-glucan viscosity factors can be altered by processing technique, cooking, storage conditions and interaction with other food components and hence these can influence the physiological functions negatively (Beer et al 1997b; Tosh et al 2010; Wang and Ellis 2014). Consequently, in terms of understanding the potential health benefits of oat incorporated food products only determining the β-glucan content, although important, will not give the full picture.

Most clinical studies examining the effect oat β glucan in products (based only on the β-glucan dosage of the test sample) show significant positive impact on health (Hblebowicz et al 2008; Charlton et al 2012; Zhang et al 2012; McGeoch et al 2013) but few clinical studies do not show any positive physiological impact with the consumption of oat incorporated products (Lovegrove et al 2000; Chen et al 2006). One of the factors for this discrepancy could be due to the variation in the MW of the solubilised β-glucan in the gut which would affect the alimentary viscosity (Wang and Ellis 2014). Some researchers have studied the impact of β-glucan properties on the physiological impact on human subjects. Wolever et al (2010) reported higher reduction of LDL cholesterol with cereal containing high molecular weight β-glucan

in comparison to low molecular weight β-glucan in subjects with high LDL cholesterol. Regand et al (2009) reported that depolymerisation of β-glucan in bread and pasta reduced the effectiveness of β-glucan to reduce the peak blood glucose reponse in healthy human subjects.

Most in vitro studies which have evaluated the β-glucan viscosity of oat products, have mostly studied the variation in β-glucan viscosity due to different processing techniques or formulations, as they have an effect on molecular weight and solubility of β-glucan (Tosh et al 2010; Gamel et al 2012; Gamel et al 2014). However, limited research has been conducted on the variations in viscosity and molecular weight of extracted β-glucan from oats, due to cultivar and environmental differences (Beer et al 1997a; Colleoni- Sirghie et al 2003). Thus, it is important to study the β-glucan viscosity of different types of oat cultivars pre and post processing techniques and understanding which oat cultivars can be easily incorporated into food products, which contain β-glucan that maintains sufficient physiological functions or health benefits associated with β glucan after processing. This will provide greater understanding of oat cultivars which are better suited for incorporation in food products.

This research area for doctoral studies was an extension of preliminary research conducted in 2011. Research work on β-glucan viscosity, solubility and molecular weight which began in 2013 resulted in key findings that contributed to the following knowledge about quality differences among Australian oat cultivars: the effect of season on oat cultivar quality; and the understanding of oat cultivar suitability for producing oat-wheat noodles with superior sensory, nutritional and health properties. In Canada, some researchers were simultaneously conducting research on the β-glucan properties of extruded products. Publications by this Canadian group assisted in the development of the present research on oat incorporated noodles and its β-glucan viscosity, solubility, molecular weight and changes to these important characteristics due to processing. The research presented in this thesis is the first research conducted on the use of Australian oat cultivars in noodles. The research findings from this research have also resulted in external funding for a project on Chinese oats.

The main objectives of this research include:

- Characterise the flour from different oat cultivars and growing seasons in terms of its physicochemical properties such as pasting properties, β-glucan content and proximate analysis, starch composition, particle size and colour.
- Evaluate the effect of incorporating oat flour from different oat cultivars and growing seasons on the quality of white salted noodles (WSN).
- Determine the physicochemical properties of β-glucan isolated from oat flour and oat enriched white salted noodles, of different oat cultivars and growing seasons.
- Evaluate the effect of processing on the susceptibility of β-glucan to breakdown of different oat cultivars and growing seasons.

Key Outcomes:

- Increased knowledge on the quality criteria needed for the identification of the Australian oat cultivars most suitable for incorporation in noodles in terms of processing ability and noodle quality.
- Establishment of methods for identification of high β-glucan viscosity oats.
- Identification of the importance of specific processing conditions on the nutritional quality of oats such as β-glucan content and its susceptibility to breakdown due to processing.
- Identification of oat cultivars that are susceptible to the breakdown of β-glucan during processing.
- Identification of the effect of production environment on selected quality characteristics of oats.

Chapter 2: Literature Review

2.1 Origin, history and classification

In 1753, a Swedish scientist, Linneaus designated *Avena* as the generic name of oats (Lásztity 1998). Although, the origin of oats is not certain archaeological discoveries trace oats back to the Greeks, Romans and Chinese in the first century and the grain may have originated in areas surrounding Mediterranean sea in the countries of the Middle East (Hareland and Manthey 2003; Zwer 2010).

Oats have been planted in China for more than 3000 years (Wang 2004a) but were initially only used as food for horses, dairy cattle and pigs in Australia and other parts of the world. Oats were not an important crop to man as early as wheat and barley. Around 2000 BC when farming developed in Europe, oats persisted as weed like plants in other cereals before they were cultivated by itself (Zwer 2004). Oats were cultivated in northern regions of Western Europe between 4500-400 BC. The cool and moist climate favoured the growth of oats in comparison to wheat and barley in these regions and oats became known as the crop adapted to less productive land (Zwer 2004; Zwer 2010). Nutritional properties of oats were identified over time, however, before the 19th century the only areas where oats formed a significant part of human diet were in Ireland and Scotland (Webster 2002). By the year 1900, oatmeal was used in breakfast cereals in USA as the local mills started milling oats and they were sold in the groceries instead of pharmacies (Webster 2002; Zwer 2004; Zwer 2010). Research conducted in 20th century further supported the early indications of the nutritional and medicinal properties of oats (Webter 2002). Today, knowledge about the beneficial nutritional and physiological effects of oat products has led to an increase in the demand for oats and their incorporation into a range of different products such as baked products, beverages, infant food etc.

The oat genus *Avena L.* (*Poaceae*) belongs to the tribe *Avenea* of the family Gramineae (Hareland and Manthey 2003; Zwer et al 2004). The species described in *Avena* form polyploidy series varies from one to three chromosome sets with a basic chromosome number of seven (Zwer 2010). Early classifications listed the common cultivated oats (hexaploid species) as *Avena sativa, Avena byzantine* and *Avena Nuda* (Coffman 1961) and commercially grown a small quantity of diploid oat, *Avena Strigosa* (Webster 2002). However, in a more recent classification (Baum 1977), the designation of *Avena*

byzantine and *Avena Nuda* was dropped and included in the *Avena sativa* taxa. This is because these species are cross fertile and extensive intercrossing by breeders, developing agronomically superior oat cultivars has further reduced the differences between them (Webster 2002). *A. byzantine* Koch. is a red-oat type adapted to grow in warmer climate as winter crops (Hareland and Manthey 2003). *Avena Nuda* is a naked (hull-less) oat, high in energy, protein and nutrition (Hareland and Manthey 2003). Although husked oats represent the majority of oat production, naked oats are gaining prominence for specialist markets (Zwer 2004) and *Avena fatua* L. is a wild oat hexaploid species with little or no economic value (Hareland and Manthey 2003). The primary species cultivated is *Avena sativa* and more than 75% of the total cultivated world oats production area is sown to this species (Zwer 2010; Coffman 1961). However, *Avena sativa* and *Avena strigosa* are also grown in some regions for animal feed and fodder (Zwer 2004; Zwer 2010). It is hypothesised that domesticated oats originated from hexaploid wild oat species (*Avena sterilis*, *Avena Fatua*, or *Avena Hybrida)* with genomes A, C, and D (Zwer 2004).

2.2 Production and cultivation of oats in the world

In terms of worldwide cereal production, oats rank sixth, following wheat, maize, rice, barley and sorghum (Ahmad et al 2014). The US department of agriculture stated that oat production has seen around 60% reduction in its world production from 1960 (56,000 kt) – 2010 (23,000 kt) (AAFC 2010). From 2010 – 2015, the oat production did not show a dramatic change and ranged between 21,000 kt – 24,000 kt (USDA 2015). The US Department of Agriculture (USDA 2014) indicated a value of 23,600 kt for the global oat production for 2013/2014 and this was around 10.6% increase in production over the 2012/2013 harvest. A decline in world production was observed from 2013-2014 harvest to 2014-2015 harvest (22,377 kt), however the oat production is predicted to increase in 2015-2016 (USDA 2015). Oats are grown in many parts of the world for use as grain as well as forage and fodder, straw for bedding, hay, haylage, silage and chaff (Ahmad et al 2014). Food uses of oats include: oatmeal, oat flour, oat bran, and oat flakes for use as breakfast cereals, and also as ingredients in other food products such as muffins, cookies, bread etc. (Ahmad et al 2014; Hellweg et al 1996; Ryan et al 2011; Tosh et al 2008; Lee and Inglett 2006; Hüttner et al 2011). Interest by consumers in oat noodles, oat rice, oat milk and oat healthcare products is growing (AEGIC 2014b).

Oat producing areas in the northern hemisphere are primarily located between 40°N and 60°N and include areas of countries like North America, Europe, and Asia (Zwer 2004). The oat growing regions in the southern hemisphere occur within the latitude 20°S and 45°S and are grown in areas of Australia, New Zealand and South America (Zwer 2004). Average oat production of the top five oat producing countries in the world over the period 1993-2013, shows that the Russian Federation and Canada are the major oat producing countries, followed by United States of America, Poland and Australia (FAOSTAT 2015).

Oats tend to grow in temperate regions (Stewart and McDougall 2014) and are either spring, winter and/or autumn sown depending on regional climatic conditions, crop rotation requirements, end use and other farming practices (Ahmad et al 2014). They are mostly grown in cool moist climates and they are sensitive to hot, dry weather (Sangwan et al 2014). Therefore, in warmer regions, spring type oats are sown in autumn to avoid summer heat and drought (Ahmad et al 2014). They usually grow best in moderate temperatures and long day length (Stewart and McDougall 2014). Oats are better adapted to grow in variable soil types in comparison to other cereal grains (Ahmad et al 2014). Oats perform better in soil with a pH of 5.3-5.7, but they can also grow in acidic soil (pH up to 4.5) (Alam and Adams 1979; Stewart and McDougall 2014). In comparison to other food cereals, they are relatively more resistant to foliar diseases and require fewer pesticides and fertiliser inputs (Givens et al 2004). So the temperate climate, winter rainfall and acidic soils of Western Australia are ideal for oat production.

2.3 Australian oat industry

Australia is a world leader in the production of safe, clean, food grade oats (AEGIC 2014a). The total production of oats for grains in Australia from 2013-2014 was around 1255 kt, which was 12% higher than the total oats produced in the year 2012-2013 (ABS 2015) as shown in Figure 2.1. However, a decline in oat production in Australia was noted in the year 2014-2015 (1096 kt) but the oat production is forecasted to increase (1300 kt) during 2015-2016 (USDA 2015). Oats are planted during May and grown through the winter months (AEGIC 2014a). Australian oats are graded for milling oats or feed purpose oats. As per Grain Trade Australia oat standards 2014/15, oats in Australia are classified as prime milling oats, milling oats and feed oats (GTA 2014). Oat varieties which can be classified as milling oats must have high hectolitre

weight with low screenings, high groat percentage and milling yield, a good level of β-glucan, low oil percentage and superior taste and aroma (DAFWA 2006; AEGIC 2014a).

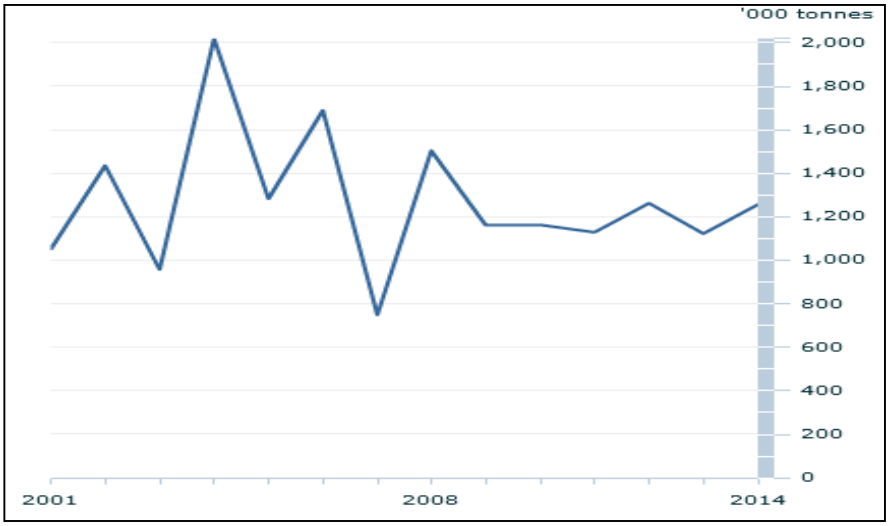

Figure 2.1 Oat production in Australia over the period 2001-2014

(Source: ABS 2015)

Milling oats are produced in the grain cropping regions of Peninsulas of South Australia (Eyre and York), western and north-eastern Victoria, the Riverina and central New South Wales, and south-west Western Australia (AEGIC 2014b) as shown in Figure 2.2 The oats produced in New South Wales and Victoria are often processed domestically (AEGIC 2014a). Western Australia (WA) grows oats to meet demand for both domestic and export markets (AEGIC 2014a). The major export markets of WA milling oats are China, Mexico, North Asia, South-East Asia and South Africa (DAFWA 2015). AEGIC in partnership with GRDC is investing in oat agronomy research in order to improve productivity, profitability and production of export quality oats so that industry can better respond to the growing demand of oats in grain food export markets such as China (DAFWA 2014).

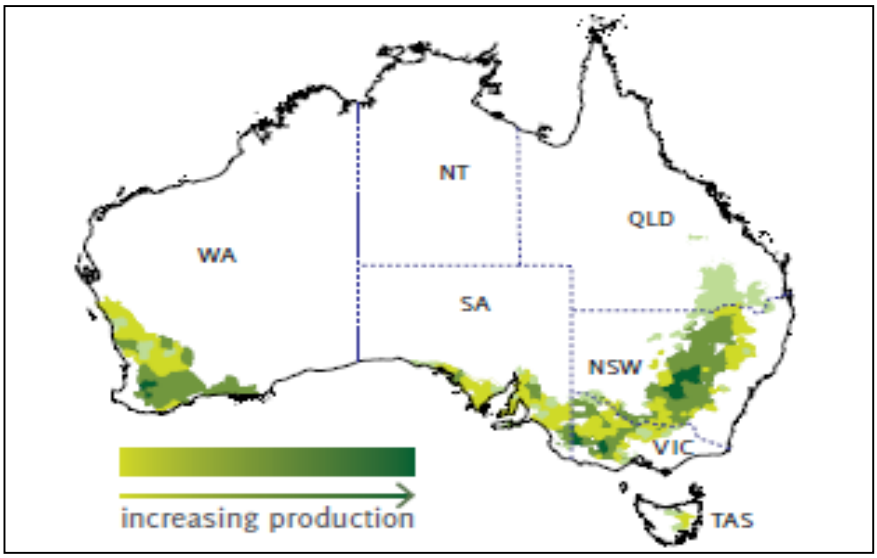

Figure 2.2 Area of oat production in Australia

(Source: AEGIC 2014b)

The total production of oats in Western Australia (WA) averaged 506 kt over the period 2009-2013 and was the highest in comparison to the other states in Australia (ABARES 2015) as shown in Figure 2.3. Oat grades from WA include Oat 1 premium milling (premium food grade milling and processing); Oat 2 standard milling (food grade milling and processing) and OWAN1 oat wandering (premium export grade for racehorse industry) (GIWA 2014). WA milling oats have an excellent reputation, both nationally and internationally for the aesthetic features of the grain (bright and plump), high groat levels and milling capabilities (DAFWA 2015). The National Oat Breeding Program for Australia has the responsibility for breeding and developing new oat varieties with superior quality (AEGIC 2014b). In Western Australia oats are an integral part of the farming system due to their rotational benefits and weed control by cutting of hay. Growing oats is also beneficial for farmers as an additional cereal crop as they tend to be less prone to frost damage and water logging (DAFWA 2006).

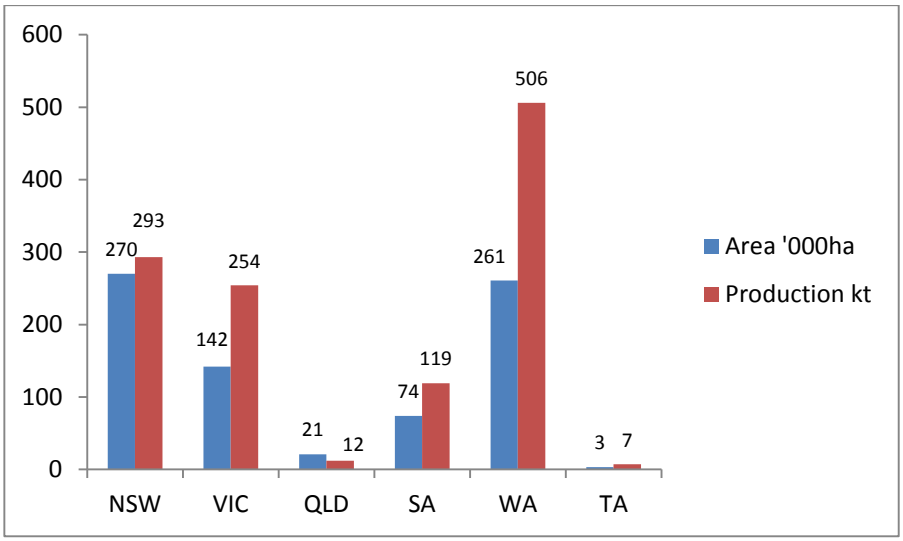

Figure 2.3 Australian state production/area of oats, averaged over the period 2009 – 2013

(Data source: ABARES 2015)

2.4 Anatomy of oat kernel

The major anatomical feature of the oat kernel (Figure 2.4) is the hull and groat (Hareland and Manthey 2003). The groat is that portion of the oat kernel after removal of the hull. The hull of the oat kernel is loosely attached to the caryopsis and makes up 30% -40% of the total grain weight and is composed of cellulose, hemicellulose and lignin (Hareland and Manthey 2003; Zwer 2004).

The oat groat/whole grain (after removal of hull) contains three morphologically distinct structures: starchy endosperm, germ and bran (Webster 2002; Sangwan et al 2014). Unlike all the other cereal grains, oat groats do not allow clean separation into these fractions due to their soft texture and the high lipid content of the grain (Webster 2002). The outer portion of oats is comprised of bran layers, which consists of the pericarp, seed coat and aleurone cells (Hareland and Manthey 2003). The aluerone layer is part of the endosperm but adheres to the outer bran layer, on separation of bran from endosperm during milling (Hareland and Manthey 2003). The endosperms cells are larger with thinner walls in comparison to the bran and high fat content of the endosperm along with high β-glucan present in the cell walls distinguish oats from other cereal grains (except for β-glucan in barley) (Webster 2002). The embryo, which is part of the groat, is made up of scutellum and embryonic axis (Hareland and Manthey 2003). The endosperm provides nutrients for the growing embryo, and the

scutellum located between embryo and endosperm helps in germination and food transfer (Zwer 2004). In comparison to other cereal grains, oat groat is slender and elliptical in shape and has trichomes (hairs) under the hull and have groove on the inner surface of the groat (Webster 2002; Zwer et al 2004; Butt et al 2008). The relative proportion of the bran layers and the endosperm is affected by both genotype and environment (Hareland and Manthey 2003). The proportion of kernel fractions from cultivated species are estimated as: hull, 24-32%; groat, 65-85%; embryonic axis 1-2%; bran layers 27-41% with a total thickness of less than 0.1mm; and starchy endosperm, 56-68% (Hareland and Manthey 2003). Oat processing and product are influenced by the unique morphology of the oat grain.

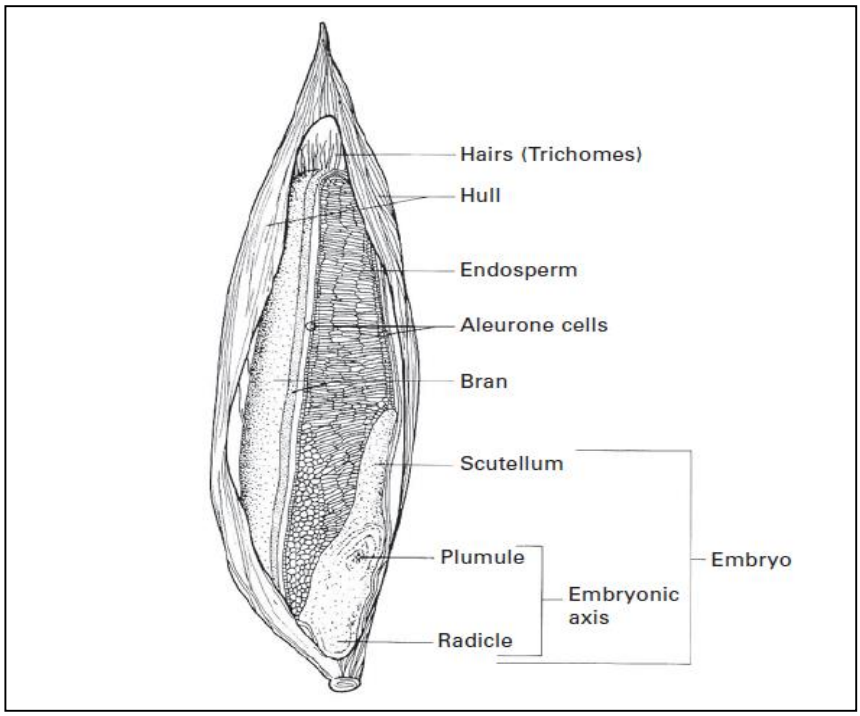

Figure 2.4 Anatomical features of oat kernel

(Source: Youngs 1986)

2.5 Biochemical and nutritional quality of oats

Oats are distinct among cereals due to their nutritional profile. In comparison to other cereals, oats contain a large amount of nutritionally superior protein, soluble dietary fibre (β-glucan), lipids with desirable balance between polyunsaturated/ monounsaturated fatty acids, and minor components with significant antioxidant activity (Webster 2002; Sangwan et al 2014). The bran of the groats contains higher concentrations of lipids, vitamins, minerals and fibre, while the endosperm has higher

concentration of digestible carbohydrate. Therefore, the ratio of bran and endosperm has an effect on the total chemical composition of the groat (Hareland and Manthey 2003).

2.5.1 Carbohydrate

The total carbohydrate content of the oat grain can range from 75-85% (Webster 2002). Starch is the major carbohydrate in oats and is primarily found in the endosperm with levels ranging from 44-61% in the oat groats (Webster 2002; Hareland and Manthey 2003). The primary components of starch are amylose (linear glucose polymer with α1-4 linkages) and amylopectin (branched glucose polymer with α1-4 linkages and α1-6 linked branch points) which are present in the ratio of 1:3 in oats, which is similar to that of wheat and corn (Hareland and Manthey 2003; Webster 2002; Zwer 2010). Oat starch exists in clusters of irregular shaped granules compared to other cereals that have distinct A- and B-type granules (Zwer 2010). Oat starch has unique pasting properties and requires a short time to peak viscosity and low pasting temperature (Zwer et al 2010). Oat starch differs from wheat starch in having a higher peak viscosity, setback and low gel rigidity (Zhou et al 1998b). In comparison to wheat and corn starch, oat starch paste has very high initial setback viscosity and shows a dramatic increase in its consistency with cooling and produces clearer gels which does not retrograde severely (Paton 1987; Paton 1977; Paton; 1979). Lower percentages of retrogradation of oat starch is related to the internally bound lipid (Wang and White 1994). Also, oat starch differs from other wheat and barley starch in showing higher leaching of amylopectin and less amylose in water dispersion and larger amylose-lipid dissociation enthalphy (Doublier et al 1987; Wang and White 1994).

Oats are also a major source of non-digestible polysaccharides, which are carbohydrates that make up dietary fibre. They consist of many substances from plant origin which cannot be digested in the human upper gastrointestinal tract (Rasane et al 2015). They include polysaccharides such as cereal β-glucan, arabinoxylans (pentosans) and cellulose (Webster 2002, Manthey et al 1999, Rasane et al 2015). Whole oats contain a significant amount of dietary fibre, especially water soluble (1-3) (1-4) β-glucan which can range from 2.2 to 8.5 g/100 g (Peterson 2001; Flander et al 2007). β-glucan accounts for at least 80% of the total neutral sugars in the soluble dietary fibre (Manthey et al 1999). The cell wall of the aleurone layer and partially the

endosperm of oat grain have high concentration of (1, 3) (1, 4)-β-glucan as they form a structural component (Würsch and Pi-Sunyer 1997; Hareland and Manthey 2003).

Dietary fibre intake from oats directly affects the gastric emptying time, rate of nutrient absorption from the intestine, faecal bulk and frequency of bowel movements (Hareland and Manthey 2003). It also indirectly helps in pancreatic hormone secretion, hepatic glucose and lipid metabolism (Hareland and Manthey 2003). Water soluble β-glucans are physiologically active and they help in the attenuation of blood glucose and blood cholesterol (Rasane et al 2015).

2.5.2 Protein

Oat groats have a high level of protein that is nutritionally superior to proteins from other cereals (Brand and Van der Merwe 1996; Petkov et al 2001; Webster 2002; Sangwan et al 2014). Oats are high in total protein content, digestibility (availability) and have a balanced composition of essential amino acid (Webster 2002). Oat groat protein content ranges from 11%-20% (Webster 2002). In comparison to all the other cereal grains, oats has the highest overall protein content and the best nutritional quality as it is higher amount of limiting amino acids lysine and threonine with 17 amino acids and an Essential Amino Acid Index (EAAI) of 94.39 (Gambuś et al 2011; Klose et al 2009). The major portions of oat protein are soluble in salt and thus are classified as globulins (80%) and a small proportion comprises of water soluble albumin (1-12%) and alcohol soluble prolamins (15%) (Sangwan et al 2014; Rasane et al 2015). Albumin and globulin are rich in lysine content; therefore oats contain a higher lysine content in comparison to other cereals and have rather lower content of glutamic acid and prolamin (Lásztity 1995). Oat has a high quality protein efficiency ratio (PER) in comparison to other common cereal grains (Webster 2002). The enzymes present in oats are proteases, maltase, α-amylase, lichenase, phenoxyacetylase and hydrolase, phosphatase, tyrosinase and lipase as summarized by Caldwel and Pomeranz (1973).

2.5.3 Lipid

In comparison to other cereal grains oats have the highest lipid content (Lásztity 1998; Webster 2002; Sangwan 2014). The percentage of lipid content in oats varied from 3.1% to 10.9% but in some high oil varieties it may be as high as 15% (Schipper and Frey 1991; Peterson and Wood 1997). In contrast to other cereal grains whose lipids

are localised in the germ, oat lipid is distributed throughout the groat (Webster 2002). Oats are unique because in addition to the predominant lipid triglycerides, oats also contain a substantial amount of other lipid classes such as: phospholipid, glycolipids, sterols and free fatty acids (Webster 2002). Oats also have a desirable balance between polyunsaturated and monounsaturated fatty acids (Webster 2002; Hareland and Manthey 2003) with a ratio of 2.2 (recommended ratio is at least 1) which is essential for cholesterol lowering diets (Webster 2002). The fatty acid composition of oat lipids is significant to the nutritional and physical qualities of oats (Zwer 2010). Oat varieties have three primary fatty acids: unsaturated linoleic and oleic acids (important for human and animal nutrition) and saturated palmitic acid, comprising 95% of the total fatty acid (Zhou et al 1998a). Linoleic acid, an essential fatty acid, is utilised in the synthesis of prostaglandins, which function to regulate smooth muscles (Hareland and Manthey 2003). Palmitic acid which is the major saturated fatty acid aids in oil stability against peroxidation (Zwer 2010). Along with lipids, oats also have a considerable amount of lipase enzyme, which can cause rancidity in and short storage life for processed products of oats (Lehtinen et al 2003).

2.5.4 Minerals and vitamins

The mineral content of oats ranges from 2-3 % (Peterson et al 1975; Sangwan et al 2014). Oat groat has considerable amount of major minerals (K, Ca, P and Mg) and trace minerals (Fe, Cu, Zn, Mn) (Peterson et al 1975).

Among the water soluble vitamins oats consist of thiamine, folate, niacin, biotin (Lásztity 1998) and the B - complex vitamins are present in the outer bran (Webster 2002).

Oat grains are relatively rich in lipids and the antioxidants protect the lipids from oxidation and are important for the storage stability of various oats products (Lásztity 1998). Oats are also a source of some of the antioxidants such as vitamin E, phytic acid, phenolic compounds, and avenanthramides are the most abundant ones and other than these flavonoids and sterols are also present (Peterson 2001). Avenanthramides has antioxidant, anti-inflammatory and anti-histamine properties and provides protection from risk of heart disease from inflammation of arteries and the development of atherosclerosis (Liu et al 2004). In oats, fat soluble vitamins E are present as isomers of tocopherols and are commonly reported as total tocols. Oats are

rich in tocopherols (2.3 mg tocopherols/100 g of grain) (Young et al 1986; Peterson et al 1995). The phenols present in oats are ferulic acid, p- coumaric acid, vanillin, p-hydroxibenzoic acid are some of the compounds detected in oats (Lásztity 1998; Dimberg et al 2005). Antioxidants protect the body from membrane damage, cancer, heart diseases and age related deterioration (Tsao and Akhtar 2005).

2.5.5 Factors influencing beneficial oat components

There have been varied results reported about the nutritive and health benefits of oats. Different factors contributing to this variability are: cultivars, soil, climate and agronomy (Stewart and McDougall 2014). Frey (1998) has reported that modern oat cultivars are more resistant and less susceptible to drought. Oat cultivar variation in grain yield, starch, oil, protein and β-glucan content and oil composition were evident in different studies on oat cultivars (Sahasrabudhe 1979; Lee et al 1997; Cervantes-Martinez et al 2001; Doehlert et al 2001; Leonova et al 2008; Newell et al 2012). Research has shown that oat grain yield, starch content and molecular weight of β-glucan is strongly influenced by environmental factors, whereas content of protein and β-glucan were equally influenced by genetic and environmental factors, and grain lipid was mostly influenced by genotype (Doehlert et al 2001; Andersson and Börjesdotter 2011). Effects of environment on protein, oil and β-glucan content of oats have been studied showing protein content is affected by fertiliser N addition and total protein content is optimised by supraoptimal applied N (Stewart and McDougall 2014). Greater environmental impact on β-glucan molecular weight (71%) than its content (42%), has been reported by Andersson and Börjesdotter (2011). Warmer temperature and dryer climate increases the β-glucan content of oats (Millers et al 1993a; Saastamoinen 1995). Higher temperature during the growing season yields a higher β-glucan molecular weight (Anket-Nilssen et al 2008). According to Sahasrabudhe (1979), environmental impact on oil content of oats was stronger than on its composition. However, some researchers noted environmental impact on oat oil composition due to broader geographical variation (Rezai and Frey 1988; Saastamoinen et al 1989). In general, warm bright spring weather and cooler summer weather without excessive rains during grain filling produces oat grains with good yield and quality (Doehlert et al 2001). More research is required to understand the impact of cultivar and environment on composition of oats.

Oat grains are rich in nutrients and unique in comparison to other cereal grains in some components such as fibre. Variation in different nutrients of oats are dependent on genotype, environment or both. However, the main component of oat grain which contributes maximum to health benefits is the highly viscous dietary fibre, β-glucan. More research is required to identify and understand the physicochemical properties of oat β-glucan and its associated health benefits.

2.6 β-Glucan

Mixed linkage (1→ 3) (1→ 4)-β-D- glucan in oats is the main constituents of the endospermic cell wall (Ajithkumar et al 2005). Mixed linkage (1→ 3) (1→ 4)-β-D-glucan ranges from less than 2% to greater than 7% in oats (Webster 2002). β-glucan forms a viscous solution in water and is not digested until it reaches the large intestine, where it is then degraded by the intestinal bacteria (Webster 2002). The high viscosity and indigestibility of β-glucan is essential for significant health benefits (Webster 2002; Anttila et al 2004). In a study by Beer et al (1997b) it was concluded that viscosity development of oat products in aqueous environment depends on the content and molecular size of β-glucan hydrated or in solution. High content and high molecular weight of β-glucan are essential for health benefits (Tiwari and Cummins 2009b). Therefore, β-glucan's health benefits are not only related to its content but also to its quality. High quality β-glucan will have a high molecular weight which will increase the viscosity development and provide related health benefits.

2.6.1 Location of β-glucan in oat grain

The (1→ 3, 1→ 4) - β -D- glucan, commonly known as β-glucan occurs in the sub aleurone and endosperm cell walls of the cereal grains (Skendi et al 2003). The endospermic cell wall of oats has multiple layers and its thick inner layer of water soluble polysaccharide, mostly consists of β-glucan and a very small amount of arabinoxylan (Miller and Fulcher 2011; Wang and Ellis 2014). The cell wall of subaleurone layer is rich in β-glucan. The inner layer of aluerone cell wall has a thin layer of β-glucan, which is surrounded by thick insoluble outer layer, making the β-glucan less readily soluble than the endospermic β-glucan (Wood and Fulcher 1978; Miller and Fulcher 2011).

2.6.2 Structure of β-glucan

“β-glucans are linear homopolysaccharides composed of D-glucopyranosyl residues (Glcp) linked via a mixture of β (1→ 3) and β (1→ 4) linkages” (Skendi et al 2003). This polysaccharide is mainly composed of β (1→3) linked cellotriosyl and cellotetraosyl units and few regions contains 4-8 consecutive (1→4) linked units (Wood et al 1991a).

The structural characteristics of β-glucan can be determined by hydrolysis of β-glucan with the enzyme lichenase, a (1→ 3) (1→ 4)-β-D- glucan-4-glucanohydrolase, specifically cleaves the (1→4)-glycosidic linkages of the three substituted glucose residues in β-glucan, yielding oligomers with different degree of polymerisation (DP) (Lazaridou and Biliaderis 2007). The major product of β-glucan hydrolysis is 3-O-β-cellobiosyl-D-glucose (DP 3) and 3-O-β-cellobiosyl-D-glucose with small amounts (5-10%) of cellodextrin like oligosaccharides (DP>5) containing more than three consecutive 4-O-linked glucose residues (Lazaridou and Biliaderis 2007).

Only a small variation in the trisaccharide-tetrasaccharide ratio has been reported for oat β-glucan and this could be due to multiple factors including differences between species, growing conditions and extraction and analytical methods (Miller et al 1993a; Wang and Ellis 2014;). There have been reports suggesting the presence of amino acid residues and the inner C-6 carbon-bound phosphomonoesters in the oat β-glucan molecules but these additional structural features have not always been observed (Autio et al 1992; Vårum and Smidsrød 1988; Ghotra et al 2007).

2.6.3 β-glucan content of oats

β-glucan content of oat cultivars investigated in different parts of the world is in the range of 1.8-5.5% of the total dry weight of the oat groat and frequent range was between 4.5-5.5% (Miller et al 1993b; Saastamoinen et al 2008; Dvoncova et al 2010). Higher β-glucan content of up to 7% has also been reported (Decker et al 2014). Among cereals, oats and barley have the highest content of β-glucan but oats generally have a higher proportion of soluble β-glucan (Lee et al 1997).

The β-glucan content of oat grains is influenced by the oat cultivars (genotype), environmental factors (location, soil type, climate- temperature/precipitation), agronomic practices (sowing, fertiliser application, irrigation and harvesting) and transportation/storage conditions (days, temperature and moisture content) (Peterson

et al 2005; Tiwari and Cummins 2009a; Martinez et al 2010; Andersson and Börjesdotter 2011).

The inheritance of β-glucan concentration is controlled by two or three gene pairs and predominantly simple additive genes (Kibite and Edney 1998). Major environmental factors that affect the β-glucan content are availability of water during grain maturation (Tiwari and Cummins 2009a). High temperature in the growing period, low soil pH and dry conditions before harvest increases the β-glucan content of oats and its grain weight (Andersson et al 1978; Saastamoinen 1995; Savin 2002). Another study by Svihus et al (1997) reported that storage of oat grain in high moisture conditions resulted in degradation of β-glucan. The β-glucan content of oat grains is also affected soil nitrogen level and precipitation (Humphreys et al 1994). In a study it was noted that, increased soil nitrogen resulting from increased nitrogen fertiliser rates increased the β-glucan content of oats and barley (Tiwari and Cummins 2009b). However, genotype and environmental factors both affect the β-glucan content of grains but the ranking of genotype is generally consistent over environment (Peterson et al 1995).

For the measurement of β-glucan two analytical methods: enzymatic method (McCleary and Glennie-Holmes 1985) and Calcofluor-FIA method (Jørgensen and Aastrup 1988) have been subjected to inter-laboratory evaluation and shown to be accurate and reliable. The fluorometric method of β-glucan analysis, also called Calcofluor method. It is based on staining of β-glucan by fluorescence enhancer and calcofluor (Vis and Lorenz 1997). The fluorochrome Calcofluor form complexes with β-glucan and exhibit an increase in the fluorescent intensity of the Calcofluor (Jørgensen and Aastrup 1988). This method is now automated and machines are available (Vis and Lorenz 1997). This method is approved by (EBC) European Brewery Convention (Munk et al 1989). McCleary and Glennie-Holmes (1985) eliminated the acid hydrolysis used in previous methods. In this enzymatic method they used a second enzyme to hydrolyse the oligossacharides formed in the first step to glucose. β-glucans are digested to beta oligosaccharides with lichenase followed by complete degradation to glucose with beta glucosidase. It was felt that this extreme specificity would eliminate interference of any other polysaccharides present in the sample and would directly target the (1→ 3, 1→ 4) - β -D- glucan McCleary and Glennie-Holmes (1985) (Vis and Lorenz 1997). A modified and improved version of this enzymatic method is available through Megazyme (Australia) Ptd Ltd. This

modified method is accepted by American Association of Cereal Chemists (AACC Method-33-22).

2.6.4 Physicochemical properties of β-glucan

The main physicochemical properties include its molecular weight, solubility and viscosity.

2.6.4.1 *Molecular weight*

The molecular weight of β-glucan in oats has been investigated in a number of studies reviewed in Lazaridou and Biliaderis (2007) and found to lie within the range of 65-3100 kDa. The variation in the molecular weight of cereal β-glucan may be due to varietal and environmental factors, different techniques used for extraction and purification, aggregation phenomenon (depends on structural features and quality of the solvent) and depolymerisation during extraction due to endogenous or microbial β-glucanase (Lazaridou and Biliaderis 2007). The molecular weight of oat β-glucan in crude extracts (3000 kDa) from whole groats or bran were estimated to be more than isolated β-glucan whose molecular weights were reported to range between 1000-2000 kDa respectively by Wood (1991). A real food matrix is very different from a mixture of hydrated gums and its physiological response will depend on the cellular structure and interaction with other components in the food matrix (Wood 1991). Åman et al (2004), has reported the degradation of oat β-glucan molecular weight in bread, pasta, tea cake and pasteurised apple juice. The molecular weight is related to the gelation properties and so at high concentration, low molecular weight β-glucan can form soft gels (Doublier and Wood 1995). So, if solutions with low molecular weight β-glucan can form gels causing aggregation and resulting in the loss of solubility (Wood 2007), then researchers need to understand this relationship.

High performance size exclusion chromatography system (HPSEC) is commonly used for determining the molecular weight distribution β-glucan (Wood et al 1991b; Beer et al 1997a; Rimsten et al 2003). In this method β-glucan is first extracted by size exclusion chromatography and then it is detected fluorometrically with calcofluor. Initially the method started as an off- line process and then was developed into an online post column detection method (Anderson 1990, Wood et al 1991b). One advantage of this method is that it does not require any prior purification step. Calibration is done with pullulan standard but this has a disadvantage of

overestimation of β-glucan due to the differences in the molecular conformation between these α linked standards and β-glucan (Wood et al 1991b; Vårum et al 1991). Sometimes purified β-glucan of known molecular weight is also used as standards but each fraction should cover a narrow range and all fractions should cover the whole range of molecular weights (Rimsten et al 2003). Sometimes retention time of the peak is also used to determine the molecular weight of β-glucan. It can give misleading results if the peak is not normally distributed or it's a polymodal peak (Rimsten et al 2003). Oat β-glucan like other natural polysaccharides has molecular weight distribution that is polydisperse. Therefore, depending upon the method used different molecular weight averages are also used to describe a polymeric material (Wang and Ellis 2014).

2.6.4.2 Solubility

The solubility of polysaccharides refers to their ability in a solid form, or contained in a solid matrix, to disperse in a liquid medium, which is often water, and forms a homogenous dispersion under specified conditions (Wang and Ellis 2014). It is sometimes used interchangeably with the word extractability (Wang and Ellis 2014). The solubility is mostly expressed as the percentage of the dissolved fraction relative to the total amount of the polysaccharide in the original solid matrix under specific conditions (such as temperature) (Wang and Ellis 2014).

The blocks of adjacent β (1→4) linkages in β-glucan have a tendency to aggregate (thus lower solubility) because along the cellodextrin (cellulose like regions) portion strong hydrogen bonds are present (Izydorczyk et al 1998). The tendency for aggregation will be proportional to the frequency and length of the cellulose regions in the polymer chains (Izydorczyk et al 1998). Blocks of two or more continuous β (1→3) linkages provide chain flexibility by breaking the regularity of the β (1→4) linkage, making the polymer more soluble (Buliga et al 1986). On the other hand, a higher ratio of cellotriosyl fragment would increase the crystalline structure in β-glucan molecule and increase the conformational regularity and thus, lower the solubility (Izydorczyk et al 1998).

Solubility of β-glucan is dependent on extraction factors such as type of solvent used, duration of extraction, temperature, pH and types of digestive enzymes used (Izydorczyk et al 2000; Wang and Ellis 2014). Solubility of β-glucan in a product can

be altered due to processing for example solubility increases in extruded and oat porridges and decreased in bread with oat bran (Johansson et al 2007; Tosh et al 2010). Depolymerisation of β-glucan initially increases solubility but if the MW reduces, further β-glucan can form insoluble aggregates (Wang and Ellis 2014). Frozen storage can also reduce solubility of β-glucan chains due to increased intermolecular interactions (Lazaridou and Biliaderis 2004)

The choice of methods used to investigate the solubility of β-glucan depends on the purpose of the study. When the main aim of an investigation is to understand the health benefits of β-glucan as a soluble fibre then the solubility properties of β-glucan under conditions close to the gut environment are highly relevant (Wang and Ellis 2014). As human and animal studies are time consuming and expensive *in vitro* methods are often preferred to be used as a pre-screening tool to evaluate the properties of β-glucan in oat products (Wang and Ellis 2014).

Any β-glucan that does not get solubilised at 37°C under physiological conditions would not contribute to the viscosity of the lumen of the gut (Tosh et al 2010). Therefore, it's important to evaluate the solubility of β-glucan in food by using in vitro methods that are designed to simulate the physiological conditions of digestion in the upper GIT of humans (Beer et al 1997b; Lebet et al 1998). The test food sample is incubated at human body temperature (37°C) with series of digestive enzymes at appropriate pH similar to the stomach and small intestine (Beer et al 1997b; Lebet et al 1998; Tosh et al 2010; Wang and Ellis 2014). Another modified method (Gamel et al 2012) recently developed directly measures the viscosity developed during the simulated digestion process using Rapid Visco Analyser (RVA). RVA is a rotational viscometer that can continuously record the viscosity of a sample under controlled temperature and shear rate conditions. This allows materials to exhibit their full range of viscous properties (Booth and Bason 2007). The solubilised β-glucan in the supernatant after centrifugation can then be quantitatively measured.

Other methods for extraction of β-glucan includes hot water extraction at 100°C using heat stable α-amylase to digest pre-gelatinized starch or use harsher chemicals like sodium hydroxide (Bhatty 1993; Saulnier et al 1994; Åman et al 2004; Rimsten et al 2003; Ajithkumar et al 2005). These methods extract a higher percentage of β-glucan in comparison to the in vitro physiological extract (Wang and Ellis 2014).

2.6.4.3 Viscosity

Sir Issac Newton defined viscosity as "the proportional relationship between the flow of a fluid and force directed on that fluid" (Dikeman and Fahey 2006).

In aqueous solutions the viscosity of β-glucan is identical to guar gum and significantly higher than psyllium (Wood 1994). Oat β-glucan is a viscous and soluble dietary fibre component (Anttila et al 2004) and has high viscosity in solutions at low concentration (Anttila et al 2004). It was reported by Doublier and Wood (1995) that oat gum solutions below 0.3 % behaves like Newtonian solutions, but as the concentration was increased beyond this point, solution developed non-Newtonian shear thinning or the polymer developed pseudoplastic characteristics (viscosity increased with increase in shear rate). Shear thinning behaviour was more pronounced as the concentration of β-glucan increased (Doublier and Wood 1995).

The viscosity of any polysaccharide is measured by concentration, temperature, shear rate and the fundamental molecular characteristics of the structure and molecular weight (Wood 1994). In order to study the physiological response of β-glucan in oat products, the major viscosity variation will occur from the amount and the molecular characteristics of β-glucan since the physiological temperature and shear rate will remain constant (Wood 1994).

As previously mentioned, the physiological effect of β-glucan is associated with viscosity. The viscosity of β-glucan in food and food digests depends on its solubility, concentration and molecular weight (Anttila et al 2004). During digestion, in the upper gut, only a portion of β-glucan from oats gets solubilised. Thus, in order to understand the viscosity of β-glucan from ingested food, it should be extracted mimicking the same conditions encountered by the food in the gastrointestinal tract (Gallahar et al 1999). Various in vitro β-glucan extraction procedures (Beer et al 1997b; Wood et al 2000; Tosh et al 2010; Wolever et al 2010) have been developed to simulate the oral, gastric and upper intestinal digestive processes. In all in vitro models the digestion temperature was always 37°C, although varying types and concentration of digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase and lipase) were used (Gamel et al 2014; Hur et al 2011). The viscosity of the β-glucan extract obtained from the in vitro method was highly correlated with the physiological effects of oat products (Tosh et al 2010; Wolever et al 2010). However, this in vitro method

was time consuming and complex (Gamel et al 2012). Gamel et al (2012) developed a simple method to measure the β-glucan viscosity of cereal products using a RVA (Rapid Visco Analyser). RVA has been used in many studies to investigate the pasting properties of oat flours and also the viscosity of oat flour slurry under different enzymatic treatments (Zhou et al 1999; Colleoni- Sirghie et al 2004; Liu and White 2011; Choi et al 2012). Gamel et al (2012) developed a method to study the viscosity of β-glucan in cereal products under controlled temperature (body temperature at 37°C), shear rate of 160 rpm (equivalent to the sheer rate of 54 sec $^{-1}$, which is similar to the shear rate occurring in the gut) (Booth and Bason 2007) and combination of digestive enzymes. This method developed by Gamel et al (2012) correlated well with the in vitro digestion protocol and it did not require any pre-digestion or sample extraction steps.

2.7 Physiological function of oat β-glucan

Oats provide valuable nutrients, and benefits for health due to its viscosity, which is primarily due to the presence of β-glucan (Figure 2.5). Evidence is accumulating that indicates that β-glucan has protective roles in preventing or delaying the onset of many chronic diseases. Different health benefits associated with β-glucan include: attenuation of post-prandial plasma glucose as well as insulin level, reduction in cholesterol, thus reducing the risk of heart disease, prevention of cancer, antimicrobial and immune effects and also promotion of weight management (see below). Ever since the health promoting potentials of oat β-glucan has been reported, several studies have been conducted on subjects with health problems. Studies have shown that viscous fibres are responsible for beneficial physiological responses in human, animals and animal alternative in vitro model (Dikeman et al 2006).

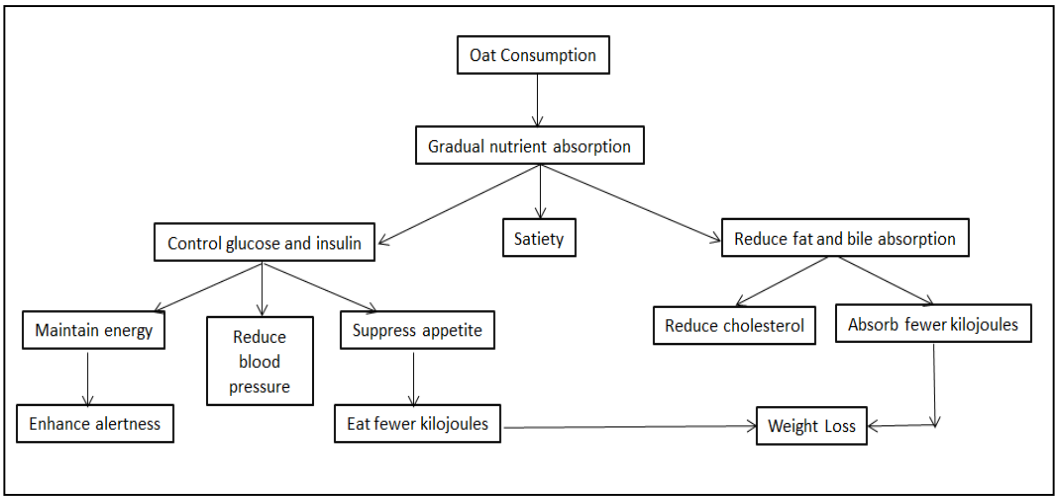

Figure 2.5 Health benefits with the consumption of oats

(Source: Zwer 2010)

2.7.1 Prevention of heart diseases

Some studies have shown that oat β-glucan has strong association with reduced risk of cardiovascular diseases (Rasane et al 2015). FSANZ (2014) and European Union health claims for barley and oat β-glucans are related to the maintenance and reduction in the blood cholesterol with consumption of 3 g of β-glucan (Harland 2014). Earlier meta-analyses (Ripsin et al 1992; Brown et al 1999) of clinical trials showed a reduction of total cholesterol by 0.13 mmol/L, with an intake of a minimum of 3 g of soluble fibre from oats. A recent meta-analysis of clinical trials (Whitehead et al 2014) showed a reduction in LDL cholesterol by 0.25 mmol/L and total cholesterol by 0.30 mmol/L, with an intake of ≥ 3 g/d of β-glucan from oats. Studies (Robitaille et al 2004; Karmally et al 2005; Reyna- Villasmil et al 2007; Theuwissen and Mensink 2007; Liatis et al 2009; Charlton et al 2012; Zhang et al 2012; McGeoch et al 2013) have indicated that consumption of β-glucan through oat based cereal products (breakfast cereals, muffins, bread, muesli bar, crisps) helps in the reduction of LDL cholesterol, total cholesterol, ratio of HDL: LDL cholesterol and increase in HDL cholesterol in male and/or female subjects with hypercholesterolemia or subjects who are overweight. However, most of the clinical studies have been focussing on the β-glucan dose effect and have not considered the viscosity or MW of β-glucan (Ames et al 2015). Clinical trials with low, medium and high MW β-glucan in extruded products and barley foods have revealed that high and medium MW β-glucan more effectively

lowered the cholesterol in subjects (Wolever et al 2010). Reduction of LDL and total cholesterol by oat β-glucan are due to several effects.

The main mechanism by which oat β-glucan has cholesterol lowering effects is dependent on its ability to entrap whole micelles containing bile acid in the intestinal lumen due to its viscosity. This excludes them from interaction with the intestinal transporters on the intestinal epithelium; this decreases the absorption and reabsorption of fats (including cholesterol and bile acid) which causes an increase in the excretion of these two components (Judd and Trusswell 1981; Marlett et al 1994; Anttila et al 2004; Rasane et al 2015). Therefore, β-glucan increases the exclusion of bile acids (Marlett 1997; Ellegård and Andersson 2007) and this in turn, activates cholesterol 7α-hydroxylase and up regulates low density lipoprotein receptor (LDLR) and thus increases the transport of LDL into hepatocytes and the conversion of cholesterol into bile acids (Nilsson et al 2007; Daou and Zhang 2012).

Another suggested mechanism for cholesterol reduction is the action of short chain fatty acids. Oat β-glucan has mixed linkages. The human small intestine lacks the enzymes that can breakdown the glucosidic bonds of the glucose molecule of β-glucan and thus, pass into the large intestine escaping digestion. In the large intestine this fermentable viscous fibre reduces enterohepatic circulation of cholesterol and bile acids (Queenan et al 2007; Wood 2007; Daou and Zhang 2012; Rasane et al 2015). The soluble fibres which enter the colon ferments completely and the end products are mainly short chain fatty acids, especially the ratio of propionate: acetate influences lipid metabolism (Daou and Zhang 2012). Research has shown that propionic acid has an inhibiting effect on liver cholesterol synthesis (Wright et al 1990; Daou and Zhang 2012). This in turn, produces the hypocholesterolemic effect (Alminger and Eklund-Jonsson 2008; Hughes et al 2008).

2.7.2 Prevention of diabetes

The European Union authorised health claims for barley and oat β-glucans are related to the reduction of post-prandial glycaemia with consumption of 4 g β-glucans/30 g of available carbohydrate (Harland 2014). Studies have shown that consumption of β-glucan through oat based cereal (oat muesli, oat bran flour product, oat meal) products can decrease the insulin levels, fasting and postprandial glucose in normal, overweight, or subjects with hypercholesterolemia, or type 2 diabetes (Tapola et al 2005; Maki et

al 2007a; Granfeldt et al 2008; Hblebowicz et al 2008). In a recent review, Tosh (2013) investigated the effect of β-glucan from oat and barley food products on the glycaemic response and considered human feeding studies using 92 processed products like bread, pasta, muffins, flakes, granola (Tosh 2013). In this review (Tosh 2013), when products containing 4 g of β-glucan was compared, 76% of the treatment showed significant reduction in the glycaemic response. The reduction in glycaemic response was highest and significant for least processed intact boiled kernel and processing procedures that resulted in higher soluble β-glucan with high MW in the products (Casiraghi et al 2006; Tosh 2013).

Oat β-glucan acts against diabetes in many ways to lower the blood glucose level. As a soluble fibre with viscous characteristics β-glucan modifies the characteristics of chyme in the upper part of the gastrointestinal tract (Daou and Zhang 2012). This reduces the mixing of the food with digestive enzymes (Marciani et al 2001; Tosh 2013) and thus, delays the gastric emptying, gut motility and nutrient absorption (Behall et al 2006), lowering the postprandial glycaemic and insulin responses (Daou and Zhang 2012). Oat β-glucan reduces the glucagon activity and this promotes the conversion of glucose to glycogen in the liver (Sangwan et al 2014). Soluble fermentable dietary fibre in the colon produces SCFAs (short chain fatty acids) such as propionic acid and butyric acid due to anaerobic fermentation by bacteria (Wright et al 1990; Queenan et al 2007). The formation of SCFAs along with an increase in the activity of P13K/Akt (prevents type 2 diabetes) by stimulating its receptors by β-glucan increases the expression of GLUT- 4 (insulin responsive glucose transporter) which favours the transport of glucose from blood into cell and reduces the blood glucose level (Song et al 2000; Brown 2006; Sangwan et al 2014).

2.7.3 Satiety and weight management

Consumption of oat β-glucan is thought to promote satiety (Slavin and Green 2007). There are several possible mechanisms. Soluble fibres delay the gastric emptying and increase the passage time in the small intestine and absorption rate of nutrients (Lyly et al 2010). These are believed to increase satiety by sending satiety – mediating signals to the central nervous system (Lyly et al 2010). The dietary fibres also form physical barriers which decrease the absorption of macronutrients and affect energy intake (Howarth et al 2001). Studies (Beck et al 2009; Lyly et al 2009) have shown that consumption of oat β-glucan increases satiety due to its viscosity and increase in

the release of cholecystokinin (appetite hormone which increases satiety). Increased satiety should decrease meal intake and consequently help in weight management (Beck et al 2009).

2.7.4 Prevention of cancer

β-glucan has been used since 1980 in immune-adjuvant therapy for cancer and tumour treatment (Daou and Zhang 2012). A study conducted by Murphy et al (2004) on mice, revealed that short term exercise training and consumption of oat β-glucan (raw oat β-glucan was fed in drinking water) decreased the metastatic spread of injected tumour cells (B16 Melanoma cells) and this was associated with an increased macrophage antitumor function against the same tumour cells in culture. Antitumor and anticancer effect by β-glucan is not solely by macrophages that attack tumour cells and destroy them, but also by modulation of lymphocyte, neutrophil and natural killer (NK) cell activity and other components of the innate immune system (Hong et al 2004). Production of short chain fatty acids from soluble fermentable dietary fibre of oats promote the proliferation of in normal colonic epithelium, retard the growth of carcinoma cell line and also induce apoptosis (programmed cell death) in carcinoma cells (Rasane et al 2015).

2.7.5 Prevention of hypertension

Controlled clinical trials on subjects with high blood pressure or stage 1 hypertension have showed an effective role of oats against hypertension after consumption of oat β-glucan or oat bran fibres for 12 weeks (He et al 2004; Maki et al 2007a). Increased body weight and insulin resistance and its associated insulinemia are major risk factors for hypertension (Ferrannini et al 1987; Neter et al 2003). Studies have shown both soluble and insoluble fibres effectively reduces insulin resistance and insulin level in both diabetes and healthy individuals and also effectively reduce body weight in overweight subjects (Rigaud et al 1990; King 2005), as discussed in the earlier sections.

2.7.6 Antimicrobial and immune effects

Oat β-glucan enhances resistance to microbial infections via cellular and antigen specific humoral immunity (Daou and Zhang 2012). It improves the immune function by increasing, in the blood, immunoglobulins, NK cells, killer T- cells, among others

and it increases the resistance to infectious and parasitic diseases (Daou and Zhang 2012).

In summary, it can be noted that consumption of oat β-glucan can have many health benefits. Although majority of clinical studies show positive impact of β-glucan on health, few clinical studies on oat incorporated products failed to show any significant positive impact on health. This could be related to the changes in the oat β-glucan structure due to processing, which reduce its functionality to improve health. Thus, it is important to understand and investigate the impact of processing on oat β-glucan structure and its related functionality for different oat incorporated products.

2.8 Impact of processing on β-glucan properties

According to (EFSA) European Food Safety Authority (2009), the health claims for β-glucan are limited to "minimally processed" foods. Health benefits are related to molecular weight and viscosity properties of β-glucan and research so far has focussed on minimally processed foods such as rolled oats. Processing may affect the molecular (chemical structure and degree of polymerisation), structural (molecular interactions) and functional properties (viscosity, water binding capacity, solubility) which in turn, can affect the sensory properties, physiological impact and thus, have a negative impact of health benefits (Brennan and Cleary 2005).

The oats used in the food industry are mostly processed into groats, flakes, flour and bran prior to incorporation in other food products or cooking by consumers (Ames et al 2015). To prevent fat rancidity the initial processing of oat grain involves heat-moisture treatment. This step inactivated rancidity causing lipase enzyme and also other enzymes such as β-glucanase (Anttila et al 2004; Ames et al 2015). Therefore, oat groats, flakes, flour and bran have virtually no endogenous enzyme activity and have intact, high MW β-glucan with high viscosity in comparison to the untreated oats (Åman et al 2004; Antilla et al 2004; Ames et al 2015;). Also, dry processing of groats such as milling, sieving and rolling does not impact the β-glucan significantly (Åman et al 2004).

Changes in β-glucan properties can occur due to high heat and shear force (Brennan and Cleary 2005). However, in extrusion processing which involves heat, pressure and shear force the solubility of β-glucan increase from 39% to 67% in breakfast cereals

prepared under mild extrusion conditions (Tosh et al 2010). The extrusion process helps to increase functionality of oat bran (Zhang et al 2011). Further increase in mechanical disruption and extrusion temperature resulted in complete solubilisation of β-glucan but the MW was reduced >10 fold. Microbial examination has revealed that severe extrusion condition causes disruption in the cell wall integrity and the β-glucan is dispersed throughout the cereal (Ames et al 2015).

In addition, when oat flour is blended with wheat or rye flour for preparation of pasta or bread, the MW and extractability of β-glucan is reduced significantly due to the action of β-glucanases in wheat and rye (Degutyte-Fomins et al 2002; Johansson et al 2007). Shorter contact time between β-glucan and β-glucanases in wheat flour and lower pH during sourdough or sponge dough bread processing (oat bran added during the dough phase) limited the extent of depolymerisation of β-glucan in comparison to the straight dough method (Ames et al 2015; Gamel et al 2015).

A decrease in the MW of β-glucan due to β-glucanase causes an increase in the solubility of β-glucan (Tosh et al 2008). In a study on oat bran muffins, it was noted that depolymerisation of β-glucan MW (from 2,200,000 to 400,000 Da) due to the β-glucanase enzyme resulted in an increase in β-glucan solubility from 44 - 57% (Tosh et al 2008). However, when the MW of β-glucan is reduced significantly (1,200,00 Da), the solubility of β-glucan decreased due to stronger self-association of depolymerised β-glucan and increased reactivity with proteins and other components found in foods (Lazaridou et al 2003; Tosh et al 2004; Wong et al 2011 Wang and Ellis 2014). Storage conditions like frozen storage affect the amount of extractable (soluble) β-glucan. The loss of solubility is due to reorganisation of β-glucan chains due to intermolecular interaction and this leads to increased ordered structure (Lazaridou and Biliaderis 2004).

2.9 Oat milling

The main aims of oat milling are to remove the inedible outer husk (crude fibre rich) from the kernel, to obtain whole oat kernels (groats) free from husks and other foreign matter, stabilise the groats and convert the groats to a form that is easy to cook and produce a product which has acceptable taste, attractive appearance, good digestibility and good shelf life (Ganssmann, and Vorwerck 1995, Decker et al 2014). The main

steps involved in milling of oats are cleaning, dehulling, hydrothermal treatment, after which they are cut, flaked or produced into flour (Figure 2.6).

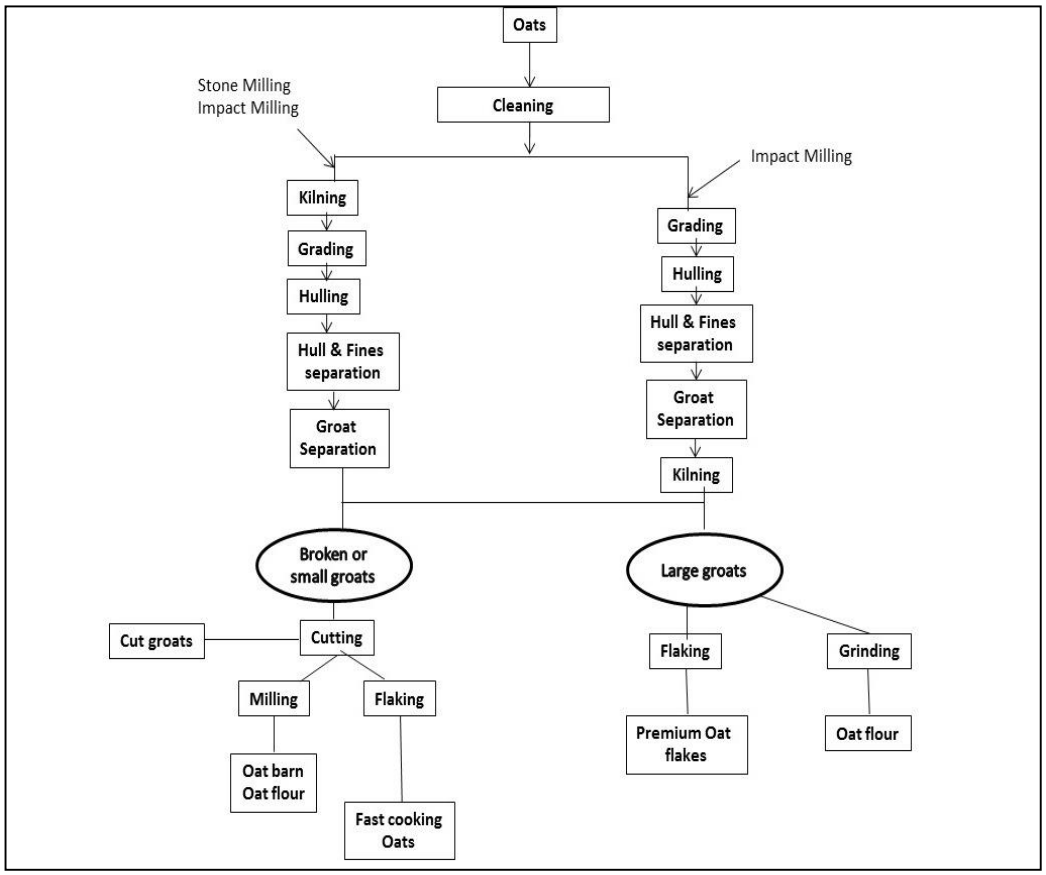

Figure 2.6 Milling process of oat kernel

(Source: Zwer 2010)

The first step of milling is **cleaning** and the oat grains are separated from co-mingled undesirable foreign matters such as straw particles, weed seeds, loose husks and foreign materials such as stones, sand and metal particles (Ganssmann and Vorwerck 1995; Girardet et al 2011; Webster 2002). The second step involves **grading**, where the cleaned oats are graded based on their physical features like density and width of the groat (Decker et al 2014). The third step involves **dehulling** of the oats to separate the indigestible husks from the groats (Zwer 2010). The impact huller detaches the groat kernels from husks by impact action and abrasion (Ganssmann and Vorwerck 1995). The oat grains are fed through a hollow shaft of the huller into the centre of a rotor (Ganssmann and Vorwerck 1995). Centrifugal force throws the oat grains against an impact ring and this impact and abrasion breaks the hull and detaches it from the groats (Ganssmann and Vorwerck 1995; Decker et al 2014). The output of the huller

can be improved by optimizing the moisture content of the grains, proportion of husk, size of the grain and motor speed (Ganssmann and Vorwerck 1995; Webster 2002; Decker et al 2014;). The fourth step is the **hydrothermal treatment** of the oat groat which is a major step in oat processing. The main purpose of this step is to achieve long shelf-life by inactivation of enzymatic rancidity and by destroying unwanted microorganisms.

Oats have very high proportion of lipids and also contain many lipases and lipoxygenases (Ovando-Martínez et al 2013). When the oat grains are damaged by grinding the lipase enzymes can act on the lipids causing the hydrolysis of the triacylglycerols and release of free fatty acids (Ganssmann and Vorwerck 1995). These free fatty acids are affected by another enzyme lipoxygenase, which catalyses the development of hydroperoxides which are then decomposed by lipoperoxidase into different hydroxyl fatty acids (Ganssmann and Vorwerck 1995, Decker et al 2014). These hydroxyl fatty acids are bitter tasting substances and are mainly responsible for the rancid, soapy taste of oat products (Ganssmann and Vorwerck 1995). As indicated above, these enzymes are inactivated by heat during processing to control this development of rancidity and off-flavour. Hydrothermal treatment using steam and heat effectively inactivate these heat labile lipid related enzyme system (Ganssmann and Vorwerck 1995; Doehlert et al 2010, Hu et al 2010; Ovando-Martínez et al 2013). The effectiveness of enzyme inactivation is usually measured by the peroxidase activity as this enzyme is more heat stable than lipase and lipoxygenase (Decker et al 2014).

After the above milling steps of oats, the whole groats are then used to make oat flakes, steel cut oats, oat flour and oat bran and in some cases even produce oat ingredients, such as fibre (Decker et al 2014). The groats or flakes are ground with a pin or hammer mill into flour (Ganssmann and Vorwerck 1995). Due to its high fat content, the oat flour tends to clump during the grinding process (Decker et al 2014). Air is used to move flour through the mill and this would decrease any heat build-up (Ganssmann and Vorwerck 1995). Oat flour is commonly incorporated in cereal based products.

2.9.1 Oat based cereal products

The utilisation of oat in many functional different foods is a recent trend. Incorporation of oats increases the nutritional properties of food products by increasing the dietary

fibre and protein content (Aydin and Gocmen 2011; Hüttner et al 2011; Mitra et al 2012). Oat based cereal products for human consumption primarily includes grain flakes and oat flour although other speciality oat based or oat derived products are also known (Hellweg et al 1996). Oat grain flakes are mainly used for preparing oatmeal, granola bars, breakfast cereals or topical additives for various products, especially bread (Estévez et al 1995; Hellweg et al 1996; Aigster et al 2011; Ryan et al 2011). Oat flour has been incorporated in extruded RTE breakfast cereals (Tosh et al 2010). Oats or oat incorporated breakfast cereals are low in calories, high in fibre and have high polyphenol and antioxidant contents. Oat components like oat bran are used for producing oat incorporated baked products like muffins and cookies (Lee and Inglett 2006; Tosh et al 2008). Oat flour has been incorporated in wheat formulations to produce oat incorporated bread and noodles (Zhang et al 1998; Flander et al 2007; Aydin and Gocmen 2011; Hüttner et al 2011; Mitra et al 2012,)

Incorporation of oats in cereal products increases the nutritional properties of the products mainly due to the β-glucan content of oats (Zhang et al 1998; Flander et al 2007; Aydin and Gocmen 2011; Hüttner et al 2011; Mitra et al 2012,). According to Wang (2004b), 50- 150 g of oats consumption would help to provide 3 g of β-glucan in the diet (which is the effective dose for reducing blood cholesterol). Incorporation of 50 -150 g of oats have an adverse effect on taste and texture and other organoleptic properties (Wang 2004b). Due to the lack of gluten in oat flour, its incorporation in wheat based products will impact its processing and sensory qualities because oat flour dough lacks gluten type viscoelasticity which hinders its utilisation in various cereal products (Zhou et al 2011). Therefore, most of the commercial oat products have low quantities of oats in their formulation (Wang 2004b).

However, it has been reported that oats can be incorporated at higher levels in noodles and still retain functionality by the addition of other ingredients. The use of more high protein ingredients (gluten, egg), addition of other additives (curdlan, transglutaminase) or improving quality through oat cultivar selection (Wang 2004b; Wang et al 2011; Zhou et al 2011; Mitra et al 2012) have been reported to improve noodle functionality. Twombly et al (2006) had also reported that the simplicity of noodles makes them an ideal base for incorporation of non-traditional ingredients such as garbanzo beans, barley, Nutrim- oat hydrocolloid, seaweed, lupin, sweet potato

starch, rye, coconut, barley fiber and oat flour (Lee et al 1998; Kruger et al 1998; Chen et al 2002; Hatcher et al 2005; Inglett et al 2005; Izydorczyk et al 2005; Chang et al 2008; Gunathilake et al 2008; Jayasena et al 2008; Aydin and Gocmen 2011; Mitra et al 2012). Thus, noodles is an ideal vehicle for incorporation of oats due to their simple processing technique, higher possibility for modifying the processing techniques or formulations, which allows incorporation of higher amount of oats and also due to its popularity in Asian and western diets.

2.10 Noodles

In Asian countries wheat flour noodles has been a staple food for years (Hou and Kruk et al 1998; Fu 2008; Majzoobi et al 2014). It is believed that noodles originated from China around 5000 B.C. and then they have spread to other Asian countries (Hou and Kruk et al 1998). During the last decade, noodle consumption has increased considerably in Western countries (Hou and Kruk 1998; Jayasena et al 2008). Wheat based noodles are mainly prepared using three basic ingredients; flour, water and salt (Fu 2008).

Noodles can be classified based on the basic ingredients (wheat flour noodles: Chinese and Japanese or Buckwheat noodles: Soba); salt used (white noodles: containing salt and yellow noodle: containing alkaline salt); size of the noodle strands (So-men & Hiya – mughi: thin noodles, served cold; and Udon & Hira-men: thick noodles, served hot) and on processing techniques (hand -made and machine made) (Hou and Kruk 1998).

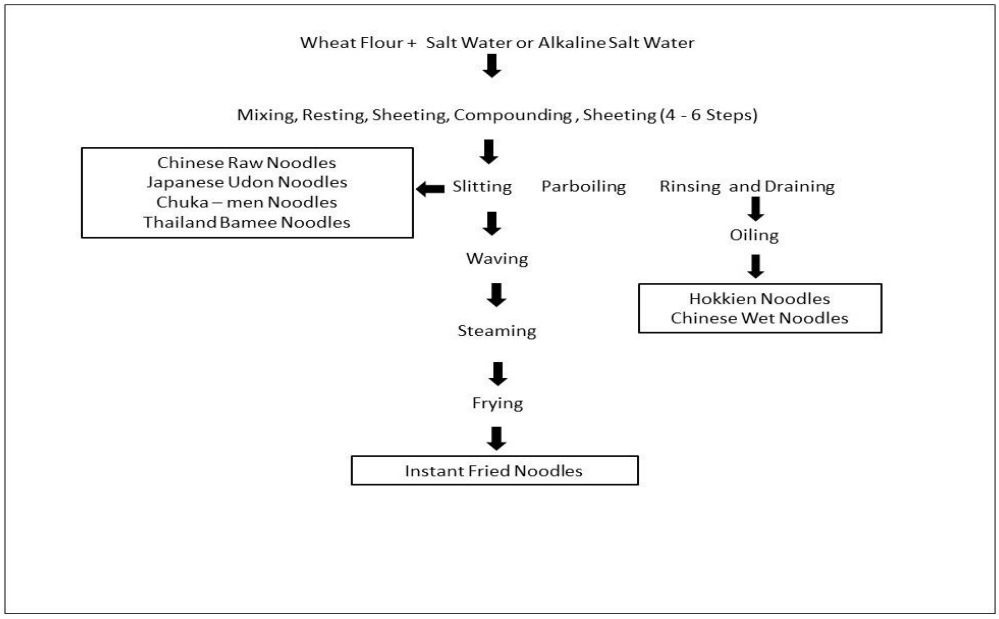

Figure 2.7 Noodle making process

(Source: Hou and Kruk 1998)

The basic noodle processing techniques for machine made noodles are shown in Figure 2.7. The first step of noodle processing involves mixing of all the ingredients in a horizontal or a vertical mixture (Hou et al 2010; Ross and Crosbie 2010). This helps in uniform hydration of flour particles and formation of a crumbly dough with small and uniform particle size (Hou and Kruk 1998; Fu 2008). Due to relatively low addition of water, the gluten is only partially developed which prevents the dough from becoming sticky and helps in formation of smooth and uniform noodle sheets (Fu 2008). The next step in the processing of noodles is the dough resting stage during which the water penetrates the dough particle evenly (Hou and Kruk 1998). The next step is sheeting between rollers. The dough is compressed between series of rolls to form a dough sheet (Hou and Kruk 1998; Fu 2008). After the mixing and resting stage, the gluten matrix is not complete and localised without continuity (Hou and Kruk 1998; Fu 2008). During this sheeting process, a continuous gluten matrix is formed which contributes to the noodle texture (Hou and Kruk 1998). The sheeted dough of desired thickness is then slit or passed through cutting rollers (strand width varying from 1.0 m to 7.5mm) to produce noodles of a particular dimension (Crosbie and Ross 2004; Ross and Crosbie 2010). These fresh noodles are either consumed within 24 hours or they are dried under the sunlight or in a controlled chamber to increase their shelf life (Hou and Kruk 1998). Fresh noodles are also either parboiled (90% cooking)

or fully boiled to produce hokkein, Chinese wet noodles or boiled udon noodles (Hou and Kruk 1998; Crosbie and Ross 2004). For instant noodles, wavy noodles strands are conveyed into steamer which gelatinise the starch and fix the noodle waves. The noodle blocks are then fried in deep fryer and made into instant noodles (Hou and Kruk 1998; Fu 2008).

2.10.1 Noodle flour quality

Each noodle type requires its own specific flour characteristics or specifications. Flour protein, ash and pasting properties are important characteristics of flour to determine noodle quality (Hou and Kruk 1998). Flour protein has a positive correlation with hardness and Japanese Udon requires soft wheat flour, 8-9.5% protein and other noodles require hard wheat flour with higher protein (10.5%-13%) which are firmer and springy in texture (Hou and Kruk 1998). Most noodle flour requires ash content below 0.5% and low ash flour produces noodles that retain clean bright appearance (Hou and Kruk 1998). Starch pasting properties can be determined by using a Rapid Visco Analyser (RVA and is dependent on the ratio of amylose and amylopectin). For Japanese noodles, flour amylose content of 22-24% is desirable (Hou and Kruk 1998). In general, for Japanese WSN, flour with high starch paste peak viscosity and high levels of paste breakdown during shear is preferred (Crosbie and Ross 2004).

2.10.2 Noodle quality

According to Jeffers et al (1979), some of the most important characteristics of noodles quality testing are: colour of raw noodle, colour of cooked noodle, texture, taste and yield. Many studies have been conducted to determine the factors such as flour particle size, starch damage, protein quality, starch pasting properties and alkaline reagents used in the formulation and their impact on textural, colour and cooking quality of noodle (Crosbie et al 1999; Hatcher et al 2002; Hatcher et al 2008)

It is preferable that all noodles are bright in colour (Hou and Kruk 1998). The colour of the noodles is preferred to be white or yellow depending upon the absence or presence of alkali salt (Hou and Kruk 1998). Also, minimal noodle darkening within 48 hours is desirable (Hou and Kruk 1998), which is dependent on polyphenol oxidase activity, weather damage, flour extraction rate and varieties (Crosbie et al 1999). In Japan, consumers prefer creamy white bright coloured noodle (Jun et al 1998; Hatcher et al 1999), whereas in China and Korea consumers prefer slightly whiter and bright

coloured noodles (Lee et al 1987; Huang and Morrison 1988 Crosbie et al 1998). Low ash and low protein content contributes in developing bright coloured noodles (Jun et al 1998). Colour of noodles and raw noodle sheet colour can be measured using spectrocolorimeter equipped with D65 illuminant and *L*a*b** (CIE 1976) colour scale (Hatcher et al 1999; Mitra et al 2012).

Eating quality is a primary criterion used to judge a noodle quality. In Japan, there is a preference for soft, elastic with slight firm and smooth surface noodles (Konik et al 1992). On the other hand, in China firmer noodle texture is sought (Crosbie et al 1998). The texture of cooked noodles can be analysed by an instrumental texture analyser (Ross et al 2006). The texture analyser helps to determine physical properties of noodles mechanically as peak force or total work (area under force/ time curve) observed during a single cutting, compression or stretching operation. This is related to the sensory property of the force required to bite a noodle strand. Texture analyser instruments can also be used to monitor the changes in the texture of noodles with any changes of raw materials, processing and formulations (Hatcher et al 1999; Ross et al 2006; Hatcher and Anderson 2007). Noodle characteristics have also been analysed through sensory evaluation (Konik et al 1993) to determine qualities like noodle smoothness, softness, elasticity and colour (Konik et al 1993).

2.10.3 Oat incorporated noodle quality

A review of research into the effect of wheat flour substitution with oat components (oat flour, oat bran concentrate, and oat β-glucan) at different level of concentration (5%, 10%, 20%, 30% and 40%) on different types of noodles (white salted, yellow alkaline, instant fried, salted dried) is presented in Table 2.1. Incorporation of oats in noodles improved its nutritional quality by increasing the protein, minerals, dietary fibre and soluble β-glucan content of the noodles as oat flour had higher percentage of these components in comparison to wheat flour (control). However a detrimental impact on noodle colour, texture, cooking quality and sensory scores was observed. Sensory panellists in some studies noted that 5% and 10% oat incorporated noodles had a closer and almost similar quality to control than the noodles containing higher percentage of oats (Reungmaneepaitoon et al 2006; Aydin and Gocmen 2011; Mitra et al 2012). Instrumental noodle CIE colour readings for oat incorporated noodles show a lower value for *L** (lightness) and higher value for *a** (redness) in comparison to the control wheat noodles. The CIE *b** (yellowness) colour value increased

(Reungmaneepaitoon et al 2006; Aydin and Gocmen 2011) or decreased (Majzoobi et al 2014; Mitra et al 2012) in some studies with the addition of oats in comparison to the control wheat noodles. Darker coloured noodles are preferred by some consumers who are aware that white-coloured cereal products are produced from ingredients that are depleted in nutrients (Izydorczyk et al 2005; Mitra et al 2012). Also, noodles like udon noodles are consumed in soups which mask the darker colour (Mitra et al 2012). Instrumental texture analysis of the noodles incorporated with oats gave a lower value for firmness and tensile strength and higher value for stickiness in comparison to the control wheat noodle (Table 2.1). The β-glucan in oats is viscous and it may hold more water and causes softening of noodles as reported by Brennan and Tudorica (2007). The cooking quality of noodles incorporated with oats had a higher absorption of water, higher solid loss and β-glucan loss (Aydin and Gocmen 2011; Mitra et al 2012; Majzoobi et al 2014). Oat replacement in wheat flour causes disruption of starch–protein matrix, dilution of gluten content and also higher levels of water soluble components in oat flour (like β-glucan) (Tudorica et al 2002; Izydorczyk et al 2005; Sabanis et al 2006; Aydin and Gocmen 2011; Majzoobi et al 2014). Zhou et al (2011), used additive like gluten to improve the texture and cooking quality of white salted noodles. Other researchers have used other additives like egg albumin, curdlan and transglutaminase in noodles for structural benefits (Wang 2004b; Wang et al 2011). Also, there remain many opportunities to improve the quality of noodles made with oat flour by ensuring selection of oat cultivars that are better suited to this purpose (Ames et al 2013). In a previous study, superior cultivars in terms of ease of processing and maintaining of β-glucan levels after cooking and processing were identified (Mitra et al 2012).

The pasting profiles (using RVA) of oat flour or meal of different oat cultivars grown in different sites over different seasons have been analysed to predict the quality of oat products and consumer acceptance (Zhou et al 1999; Choi et al 2012). However, only limited work (Mitra 2012) has been done to investigate the effect of different oat cultivars grown in different seasons, on noodle quality (texture, colour, cooking quality of noodles).

Table 2.1 Studies on wheat flour substitution by oat components in noodle products

Product	Oat fraction incorporated	Other Additives/ ingredients	% of oats added	Composition of oat noodle/oat–wheat blend/ oat flour[a]	Instrumental CIE Colour measurement[a]	Instrumental Texture measurement	Cooking Test	Sensory Analysis	Reference
Salted Dried Noodles	Oat flour	Salt	10, 20, 30, 40	Oat flour had ↑ Protein ↑ Crude fat ↑ Ash ↑ TDF In comparison to control wheat flour	↓ CIE L* ↑ CIE a* ↓CIE b*	↓ Cutting force In comparison to the control	↑ Water Absorption ↑ Cooking loss In comparison to control	Ranking Test (1-0 = very bad; 4-5 =excellent): Significantly ↓ taste scores for 30% and 40% oat incorporated WSN in comparison to the control Significantly ↓ colour and texture scores for 40% oat incorporated WSN in comparison to the control	Majzoobi et al 2014
White Salted Noodles	Oat flour (5 different WA cultivars of oats)	Salt	10, 20, 30	Wheat-oat blends had- ↑ Protein ↑ Ash ↑ β glucan In comparison to the wheat flour control Oat Noodle had – ↑ β glucan	↓ CIE L* ↑ CIE a* ↓CIE b*	↓ Firmness in comparison to wheat noodles	Mean loss of β glucan (g/100g) from wheat-oat blend varied between cultivars and control after processing into cooked	Consumer preference /Liking test (1 =dislike extremely; 10= like extremely) 10% oat incorporated WSN received the highest score followed by 20% and 30% oat incorporated WSN For 30% oat incorporated WSN colour and	Mitra et al 2012

				In comparison to the wheat noodle control			noodles and was highest for Yallara cultivar	appearance was disliked significantly more than 10% and 20% oat incorporated WSN colour	
White Salted Noodles	Oat flour	Pasteurised liquid Egg; sodium stearoyl-2-lactylate and salt	10, 20, 30, 40	Oat noodle had - ↑ Protein ↑ Crude fat ↑ Minerals ↑ Ash In comparison to control wheat noodle	↓ CIE L* ↑ CIE a* ↑ CIE b*	ND	↑ Water Absorption ↑ Cooking loss In comparison to control	9 point hedonic scale (1= dislike extremely; 9= like extremely): ↓ scores for taste , odour, mouthfeel, surface and chewing property and overall acceptability in comparison to control 10% oat incorporated WSN received highest scores and statistically similar to control	Aydin and Gocmen 2011
White Salted Noodles	Oat flour	Tapioca Starch Gluten (1.8%, 3.6%, 6.9%, 10%)	50	Oat flour had - ↑ Protein ↑ Ash In comparison to wheat flour control	↓ CIE L* ↑ CIE a* ↑ CIE b* In comparison to control wheat noodles	Gluten addition ↑Stretchability ↑ Elasticity ↑ Firmness ↓ Adhesiveness In comparison to the oat noodle without gluten	Gluten addition ↓ Cooking yield ↓ Cooking loss In comparison to oat noodles without gluten	9 point hedonic scale (1= dislike extremely; 9= like extremely): Gluten addition in oat flour causes: ↑ scores for appearance, taste and texture	Zhou et al 2011

Yellow Alkaline Noodles	Oat β glucan (Nutrim)	With 30% Banana flour (BF)	10	BF and oat noodles ↑ Crude Protein ↑ Crude fibre ↑ Crude fat ↑ Minerals ↑ Ash ↑ Resistance starch In comparison to the wheat flour control	ND	ND	ND	9 point hedonic scale (1= dislike extremely; 9= like extremely): ↓ scores for firmness, elasticity, surface smoothness and flavour in comparison to noodles not incorporated with oat β glucan	Choo and Aziz 2010
Instant Fried Noodles	Oat bran Concentrate (OBC): XF (coarse flour), XEF (Fine flour), native	Salt and Alkaline solution	5, 10, 20 XF, XEF and Native	Oat flour and noodles had - ↑ Protein ↑ fat Presence of β glucan In comparison to control wheat flour/noodles	↓ CIE L* ↑ CIE a* ↑ CIE b*	↑ Stickiness ↓ Tensile strength Significantly ↓ firmness for 10% and 15% OBC XF/XEF In comparison to control	ND	9 point hedonic scale (1= dislike extremely; 9= like extremely): Elasticity , texture and acceptability of 5-10% OBC XF,XEF, native was statistically ↔ as control	Reungmaneepaitoon et al 2006
Yellow Alkaline Noodles and White Salted Noodles	Oat β glucan (Nutrim)	30%, 40%, 50% rice flour	10	↑ soluble β glucan in comparison to wheat : rice flour blends	ND	Addition of Nutrim gave satisfactory results for tensile strength even with 50% rice flour	Addition of Nutrim gave satisfactory results for cooking loss even with 50% rice flour	9 point hedonic scale (1= dislike extremely; 9= like extremely): No difference in taste between these noodles with different rice flour %	Inglett et al 2005

[a]In comparison to control noodle/flour; ↑ Higher; ↓ Lower

Further research is required to understand the variation in noodle quality due to incorporation of different oat cultivars grown in different seasons. This will help in selection of superior oat cultivars and reduce the requirement to use additives to improve the noodle quality of oat incorporated noodles. Special emphasis in understanding the oat cultivar variation in loss and degradation of β-glucan during processing of noodles is required as the β-glucan content and the extent of degradation are important factors that relate to the health benefits of oats.

Chapter 3: Materials and Methods

3.1 Western Australian oat selection

Milling oat cultivars from Western Australia (WA) were used for this study. The selected six oat cultivars from WA were: Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams. These six oat cultivars were collected over two different growing years (2011 and 2012) from the same location (Katanning, WA). Oat cultivars were supplied by the National Oat Breeding Program managed by the South Australian Research and Development Institute (SARDI), in collaboration with the Department of Agriculture and Food, Western Australia (DAFWA). The oat cultivars were grown in 70 m multiplication strips at Katanning. There was no replication. The fertiliser was Agstar at 120 kg/ha. The soil type was sandy loam over clay.

Table 3.1 Oat cultivar description

Cultivar	Description	Released
Carrolup	A widely sown cultivar as premium milling cultivar. It is tall variety with lower yields then the new cultivars	Released in 1993 by DAFWA
Kojonup	The first dwarf variety accepted as milling oats in WA. Excellent grain quality, large seeds size, high groat percentage and hectolitre weight, good grain brightness and protein and low screenings.	Released in 2005 by DAFWA
Mitika	Early maturing dwarf milling oat variety. It is an improvement on Carrolup. It has higher levels of β-glucan than the current cultivars. Mitika is moderately resistant to leaf rust. It also has high hectolitre, low screenings and high groat percentage.	Commercialised in 2005 by Heritage Seeds.
Yallara	A medium –tall milling variety. Yallara has excellent grain quality. It has high hectolitre weight, low screenings and high groat percent. It is leaf rust resistant.	Released in 2008 by the National Oat Breeding Program through SARDI (South Australian Research and Development Institute)
Bannister (New Cultivar)	A dwarf milling cultivar with high grain yield. It has slightly lower hectolitre weight and slightly higher screenings compared to Mitika,	Released in 2012 by the National Oat Breeding Program through DAFWA.

	It is similar to Mitika for groat percentage. Improved stem and leaf rust resistance.	
Williams (New Cultivar)	A tall milling variety. The grain quality is slightly lower than Mitika. Has higher screenings than Mitika, Yallara and Bannister in low rainfall regions. Good disease resistance.	Released in 2013 by the National Oat Breeding Program through DAFWA.

Source: GIWA 2014; Hoppo and Zwer 2014; Zwer et al 2015

Selected oat cultivar (10 kg of each) was stored in the freezer. The wheat flour (control) was standard commercial udon flour from Japan.

3.2 Sample preparation

For processing oat grains into oat flour, 5 kg of oat grain samples were subsampled from 10 kg of oat grains stored for each oat cultivar. The oat grains were processed into oat flour using the following steps:

1) Dehulling - Oat grains were dehulled using an air pressure (80 psi) dehuller (Model no. 5095, Codema Inc. MN, USA). 5 kg of oat grain sample for each cultivar was dehulled (50 g portion of oat grain sample was dehulled for 2 min in the dehuller).

2) Cleaning - Dehulled oat groats were cleaned in an aspirator. In the aspirator chamber (S.K. Engineering and Allied Works, India, No. SK/LAB/05/ASP) light impurities like hulls and dust were removed from the groats by means of air. Heavier impurities like stones and grains with intact hull were removed by hand picking after visual inspection.

3) Steaming and Drying – The dehulled and cleaned oat groats were heat treated within 2 h of dehulling. Oat groats are rich in lipids and heat treatment is essential to inactivate lipase or lipoxygenase enzymes as they can hydrolyze the oat lipid and develop rancidity (Ganssmann and Vorwerck 1995, Decker et al 2014). Hydrothermal treatment using steam and heat effectively inactivates this heat labile lipid related enzyme system (Ganssmann and Vorwerck 1995; Ovando-Martínez et al 2013; Doehlert et al 2010). The effectiveness of enzyme inactivation was measured by a qualitative peroxidase activity test by an approved method 22-80.01 (AACC International 2010), as the peroxidase

enzyme is more heat stable than lipase and lipoxygenase , it is used as an indicator enzyme (Decker et al 2014). The cleaned oat groats were steamed in a pressure less convection steamer (Model No. CS/E-5, Curtin, Australia) to deactivate the lipase enzyme. Temperature of the steamer set at 100°C for a time period of 30 min was required to inactivate the peroxidase activity in the groats. The steamed groats were then oven dried at 65°C for 45-60 min. The dried groats were then removed from the oven and cooled to room temperature.

4) Milling – Rotor beater mill was used to mill oat groats. The dried groats were milled to oat flour (whole groat flour) in a Retsch mill (SR 300, Retsch GmbH, Haan, Germany), using a 250 µm sieve.

The milled oat flour samples were then sealed in airtight, snap and lock plastic bags and stored in the freezer (-10°C) until use.

Figure 3.1 Equipment used in the preparation of oat grain for milling

The processing method for oat grains to flour was developed over months and the step involving the heat treatment to deactivate peroxidase activity was standardised after various trials. Initially, different temperatures (70°C, 85°C, 100°C and 110°C) and time profiles (15 min, 20 min, 25 min, 30 min, 40 min, 45 min and 60 min) were used to steam the oat groats and the steamed groats were then milled and tested for peroxidase activity. The standard processing time and temperature profile typically used in DAFWA for oat groats was: temperature of the steamer is initially set at 100°C. After the steamer gets heated then the temperature of the steamer is reduced to 70-40°C and the groats are then steamed for 12-15 min and dried at 65°C for 40 min approximately (*as per verbal communication with Max Karopoulos of DAFWA in the year 2012*). The oat cultivars grown in 2011 however required higher temperature of 100°C for 30 min to inactivate its peroxidase activity and this could have been due to very high total rainfall (668.4 mm) received during the year of 2011 with annual mean maximum temperature being 22.5°C (Bureau of Meteorology 2013) (Refer to *Appendix 1 and 2* for further details) which could have increased the peroxidase activity of the grains (AWB 2014). Although the oat cultivars grown in 2012 received lower total rainfall (481.0 mm) with annual mean maximum temperature being 23.1°C (Bureau of Meteorology 2013) (Refer to *Appendix 1* and 2 for further details), and could have been processed at a lower temperature and time to deactivate its peroxidase activity, they were treated the same as the oat cultivars grown in 2011 which successfully deactivated its peroxidase activity. The same treatment of oat cultivars for both the growing seasons was essential because they had to be compared in this study and different treatments for both seasons would add a variable.

All analyses in the following sections were performed in duplicate unless otherwise indicated.

3.3 Oat groat and flour quality characterisation

3.3.1 Oat groat quality

Assessment of oat groat colour and lipid content were the two quality tests performed on whole oat groats.

The colour of the groats was measured, in CIE *L**, *a** and *b** colour space units using a Minolta Chroma Meter (model CR310, Konica Minolta) with glass light projection tube attachment (CR-A33e). The Minolta Chroma Meter was calibrated against the supplied calibration plate before measuring colour. A subsample of 70 g of oat grains

was dehulled using an air pressure (80 psi) dehuller (Model no. 5095, Codema Inc. MN, USA) and cleaned by an aspirator and by hand picking. The clean groats were then placed in a plastic container and measured to a depth of 2.5 cm which was determined to be sufficient to remove any effect of the background on the grain colour reading. The Minolta measuring head was pressed on the groats and the reading was taken.

The lipid content of oat groats was determined by NMR (Nuclear Magnetic Resonance) (Newport, Oxford, UK). NMR was calibrated with vegetable oil. Oat groat samples were loaded in tubes and placed in the NMR machine then the measured quantity of the lipid was displayed on the screen.

3.3.2 Oat flour quality

Oat flour quality was characterised based on the chemical and physical properties. Table 3.2 outlines the range of tests used to assess the chemical composition of oat flour.

Table 3.2 Quality tests on oat flour samples

Quality Tests	Method	Principle	Instruments
Moisture content	Electrical conduction calibrated with AACC Method 44-15A, *Air Oven Method.*	Based on the ability of a sample to conduct electricity based on its moisture content.	Marconi moisture meter, model 933C (Marconi Instrument Ltd.)
Protein content	AACC Method 46-30.01, *Dumas combustion method*	Quantitative determination of nitrogen content in a sample and then multiplying it with 6.25 factor to get total protein content.	Leco Elemental rapid N cube
Ash content	AACCC Method 08-01.01, *Basic Method*	The incombustible inorganic residue of the flour sample is the ash content.	Muffle Furnace (Thermolyne, 4800)
Damaged starch content	Assay procedure for Megazyme International Ireland Ltd. Based on AACC method 76-31, *Determination of Damaged Starch-Spectrophotometric Method*	The damaged starch granules are hydrated and hydrolyzed to maltosaccharides plus α limit dextrin when treated with purified fungal α-amylase. The reaction is terminated with dilute sulphuric acid and then treated with purified amyloglucosidase which degrades all the starch derived dextrins to glucose.	Spectrophotometer (Pharmacia Biotech, Novaspec II)

		Glucose is then measured by GOPD[1] reagent mixture.	
Amylose/ Amylopectin assay	Assay procedure for Megazyme International Ireland Ltd. Based on the Gibson et al 1997 method, *A Procedure to Measure amylose in cereal starches and flours with Con A.*	The ratio of amylose content of starch in oat flour samples were analysed by precipitating and removing the amylopectin with Concanavalin A (Con A).	Spectrophotometer (Pharmacia Biotech, Novaspec II)

[1] Glucose oxidase/peroxidase reagent

In addition to the above quality tests other quality tests on oat flour were conducted including β-glucan content, particle size distribution, flour colour and pasting properties.

The analysis of (1-3) (1-4) - β - D glucan in the oat flour samples was conducted using the mixed- linkage (1-3) (1-4) β-glucan assay kit using the procedure to analyse β-glucan in oat and barley flour and fibre samples (Megazyme International Ltd., Wicklow, Ireland). This assay is based on the principles of approved method 32-23.01 (AACC International 2010). Oat flour samples (120 mg) were weighed in glass centrifuge tubes (17 mL capacity). The tubes were tapped to ensure all of the samples fells to the bottom of the tubes. 0.2 mL of aqueous ethanol (50% v/v) was added to the tubes to aid dispersion, before sodium phosphate buffer (4 mL, 20 mM, pH 6.5) was added and the content was stirred in a vortex mixer. After mixing, these tubes were immediately placed in a boiling water bath and incubated for 60 sec. The tubes were then vortexed vigorously and incubated at 100°C for 2 min and vortexed again. When the sample reached 50°C, the tubes were then equilibrated at 50°C for 5 min. Lichenase (0.2 mL, 10 U) enzyme was added to the tubes and the tubes were vortexed. The tubes were sealed with parafilm and incubated for 1 h at 50°C in a shaking water bath with regular vigorous mixing (3-4 times) with a vortex mixer. After incubation, sodium phosphate buffer (5 mL, 200 mM, pH 4) was added and the tubes were mixed vigorously with a vortex mixer. The tubes were then equilibrated at room temperature for 5 min and centrifuged (1000 g, 10 min). In 12 mL capacity test tubes, 0.1 mL aliquots were accurately dispensed into the bottom of three test tubes for each sample. β-glucanase (0.1 mL, 0.2 U) in 50 mM sodium acetate buffer (pH 4) was added to two of the sample test tubes (reaction tubes). 50 mM acetate buffer (0.1 mL, pH 4) was

added to the third sample test tube (the reaction blank). All three test tubes for each sample were then incubated at 50°C for 10 min. After incubation, GOPD reagent (3 mL) was added to all sample test tubes and incubated at 50°C for 10 min. The test tubes were then removed from the water bath and the absorbance of the samples was measured at 510 nm in a Spectrophotometer (Pharmacia Biotech, Novaspec II) within 1 h. With each set of analysis, D-glucose standard (100 μg in 0.1 mL) and oat flour standard flour sample of known β-glucan content was included. Based on the absorbance values, β-glucan content (g/100 g) of flour samples was then calculated on a dry weight basis.

The particle size distribution of the flours was analysed by laser light scattering using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). Five g of sample was dry-dispersed into the apparatus using a Scirocco 2000 dry powder dispersion unit (Malvern Instruments Ltd, Malvern, UK). Particle size distribution curves were generated through the associated software program which used volume (%) data of particle size at various ranges (4 -1000 μm). The volume (%) of particle size, below 100 μm and above 100 μm was calculated for all cultivars, and for the two growing years. 50 -100 μm was the point at which the two modes were divided. Since the range of volume (%) of particle size was from 4-1000 μm, any particle size of flour outside this range was not calculated by the software. Therefore, the total volume (%) of particle size of oat flour samples was above 98% and not 100%.

The samples for analysing the colour of the oat flour fraction were prepared according to the following procedure. Oat flour samples (10 g) were sieved through a laboratory test sieve (Aperture: 100 μm; Ser. No. 6288567, Endecotts, London). The oat flour fraction which passed through the sieve was packed in zip lock bags and labelled as oat flour fraction <100 μm and the oat flour fraction which did not pass through the sieve was packed separately and labelled as oat flour fraction >100 μm. The oat flour and oat flour fractions (>100 μm and <100 μm) CIE L^*, a^* and b^* colour was measured using a Minolta Chroma Meter (Model CR-400, Konica Minolta) with the standard narrow light projection tube attachment. The samples were mixed thoroughly and sub samples were placed in the flour analysis attachment cell (TC 189-0564F; Technicon Instruments Corporation) and levelled off. The measuring head of Minolta Chroma Meter was placed on the measuring area of the attachment and the colour readings were recorded.

A Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used to determine the pasting properties of oat flour with 20 min pasting profile (Zhou et al 1999). The test sample flour was mixed with deionised water in the RVA canister (flour weight: 3.5 g and water amount: 25 mL was corrected to 14% moisture basis). The canister was then placed in the RVA and an initial speed of 960 rpm further mixed the sample for 10 sec and then a speed of 115 rpm was used for the remainder of the test time. The temperature was controlled for the test and followed a set profile: samples were held at 40°C for 50 sec and then heated to 90°C for 3 min and held at 90°C for 6 min 30 sec and then dropped to 40°C for 4 min 30 sec and then held at this temperature for another 5 min. The peak viscosity, time to peak viscosity, trough, breakdown (peak viscosity minus trough), pasting temperature, setback, and final viscosity were recorded. Values reported were in minutes (min), degrees Celsius (°C), and centipoise (cP).

The control (udon) wheat flour samples were also included, with the oat flour samples, for analysing moisture content, protein content, ash content, β-glucan content, particle size distribution and CIE *L** *a** and *b** colour measurements using the standard methods as mentioned above.

3.4 Oat-wheat white salted noodle (WSN) processing and quality

A previous study conducted showed that a maximum addition of 30% oat flour to the WSN was possible without addition of additives or change to the processing methodology (Mitra et al 2012). A blend of 30% oat flour in wheat flour was prepared and the mixture was homogenised for each oat – wheat flour samples. These blends were then used for noodle preparation and other analyses.

3.4.1 30% oat-wheat flour blend and udon flour quality

The two quality tests performed on the 30% oat-wheat flour blend (30% OW) and udon wheat flour (control) were the determination of flour pasting properties and flour β-glucan content.

For wheat flour and oat-wheat flour blends (oat-wheat; 30:70) a rapid (13 min) protocol to evaluate flour pasting properties (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used following AACCI Approved Method 76-21.01 (2010). The test sample of flour was mixed with deionised water in a RVA canister (flour weight: 3.5 g and the water amount: 25 mL added were corrected to

12% moisture basis). An initial speed of 960 rpm further mixed the sample for 10 sec and then the speed was held at 160 rpm for the remainder of the test time. The temperature was slowly increased and decreased and followed a profile in which samples were held at 50°C for 1 min, heated to 95°C for 3.43 min, held at 95°C for 2.7 min, and then dropped to 5°C for 3.88 min. Peak viscosity, time to peak viscosity, trough, breakdown (peak viscosity minus trough), pasting temperature, setback, and final viscosity were recorded. Values reported were in minutes (min), degrees Celsius (°C), and centipoise (cP).

The analysis of (1-3) (1-4) - β - D glucan in the control wheat flour, oat flour and 30% oat-wheat flour samples was conducted using the mixed- linkage β-glucan assay kit (Megazyme International Ltd., Wicklow, Ireland) and followed the procedure to analyse β-glucan in oat and barley flour and fibre samples. This assay is based on the principles of approved method 32-23.01 (AACC International 2010). The detailed method is discussed in section 3.3.2.

3.4.2 Oat -wheat WSN (udon) processing

The official Japanese noodle (white salted noodles- udon) processing steps used by the Australuan Export Grains Innovation Centre (AEGIC) (Refer to *Appendix 3* for further details and Figure 3.2) was used for this research with some modifications for oat-wheat noodle formulation (Mitra et al 2012).

Table 3.3 Steps involved in WSN (udon) noodle processing

Stage Number	Stage	Description
1	Weighing	a) 100 parts flour (400 g mix) - 30% wheat flour replaced with oat flour to produce oat-wheat noodles; for control sample - 100% wheat flour was used (Weight of flour adjusted on a 13.5% moisture basis, Appendix 4), 34% of water (adjusted for flour moisture content, Appendix 4) and 2% (8 g) salt. b) Flour weighed into a Hobart mixure bowl and water (at 27°C) weighed into a 500 mL conical flask. Salt was added to the water and dissolved by shaking.
2	Mixing	The mixing was done in a Hobart mixer (N50). a) The flour was initially mixed for few seconds in the Hobart mixture at speed 1 (low speed), then the salt water was trickled down from the side wall of the Hobart mixture into the flour for 30 sec.

		b) The dough was then allowed to mix for another 1 min. The mixer was then stopped and any dough adhering to the bottom of the bowl or the beater was scraped back into the mix and the dough was mixed at speed 2 (high speed) for 1 min. c) The mixer was stopped and the speed was changed back to speed 1 (low speed) and mixed for another 3 min. d) End of the mixing, a dough crumb was formed with a temperature of 26°C approximately.
3	Sheeting	a) Sheeting was done on an Ohtake noodle machine (Ohtake, Japan) after warming up the machine to 27°C. b) Sheeting was done between rollers with initial roller gap set at 3 mm. The dough crumb was placed on the rolls and spread evenly and sheeted. Any tailing was gathered in the middle of the sheet, folded and passed through the roller the second time. Again the sheet was folded and passed through the rollers the third time.
4	Resting	a) The sheet was then rolled around a plastic roller (kept in the Contherm cabinet), placed in a large plastic bag and air expelled from the bag as it was rolled up. The labelled plastic bag (sample number, current time) was kept in the Contherm cabinet for 1 h at 25°C)
5	After Resting	a) After resting period of 1 h the sheet from the Contherm cabinet was further reduced in thickness by passing between rollers with 2.5 mm gap, followed by passing through the rollers again after reducing the roller gaps to 2.2 mm gap. b) The noodle sheet was then placed on a clean bench top. The thickness of the sheet was measured with a Peacock gauge No. 2 along each sides of the sheet. The average thickness was recorded. Small pieces from the sheet were cut and passed through the adjusted roller gap to get a sheet with 2.5 mm thickness Noodle sheet samples for visual analysis (not passed through rollers) and colour- Minolta analysis (passed through the rollers) were collected
6	Cutting	a) Once the rollers were adjusted to give a sheet of 2.5 mm thickness and after collecting samples for other analysis, the entire sheet was passed through the set roller gap into the udon cutter. b) The long strand of noodles were then spread on a clean bench top and cut into 12 cm length.
7	Packing	a) The noodles strands were packed in zip lock bags and used for further analysis.

Noodles were stored in plastic bags at ambient temperature before cooking (within 1 h of processing), to prepare samples for colour analysis, solid/β-glucan loss in cooking water and β-glucan content of cooked noodles followed by cooking for texture analysis. In cutting step 6 in Table 3.3 dusting of noodles with starch before cutting is done in the standard AEGIC method. This step was omitted because it would have interfered with the investigation of percentage of solid loss in noodle cooking water and β-glucan content.

3.4.3 Cooking of 30% OW noodles and control wheat noodles

Cooking loss, β-glucan content and colour analysis used a 25 g (5 cm) portion of noodles (Approved Method, 66-50.01 AACCI 2010) and for texture analysis a 7 g (7.5 cm) portion of noodles (Sissons et al 2008) was boiled in a glass beaker containing 250 mL boiling water. The level of boiling water was maintained at 90% of the original volume throughout the cooking process and the beaker was partially covered with a watch glass. The noodle samples were stirred to make sure the noodle strands were separated. Every 15 sec interval, three strands of noodles were removed and pressed between glass slides to determine the time required for the disappearance of the centre core of the noodle strands or optimum cooking time. (OCT) (Solah et al 2007; Sissons et al 2008, Approved Method, 66-50.01 AACCI 2010).

3.4.4 Noodle quality analyses

The 30% OW noodles and control udon noodles were analysed to determine the β-glucan content of the noodles, loss in cooking water (cooking loss of solids and β glucan), firmness and colour of the cooked noodles.

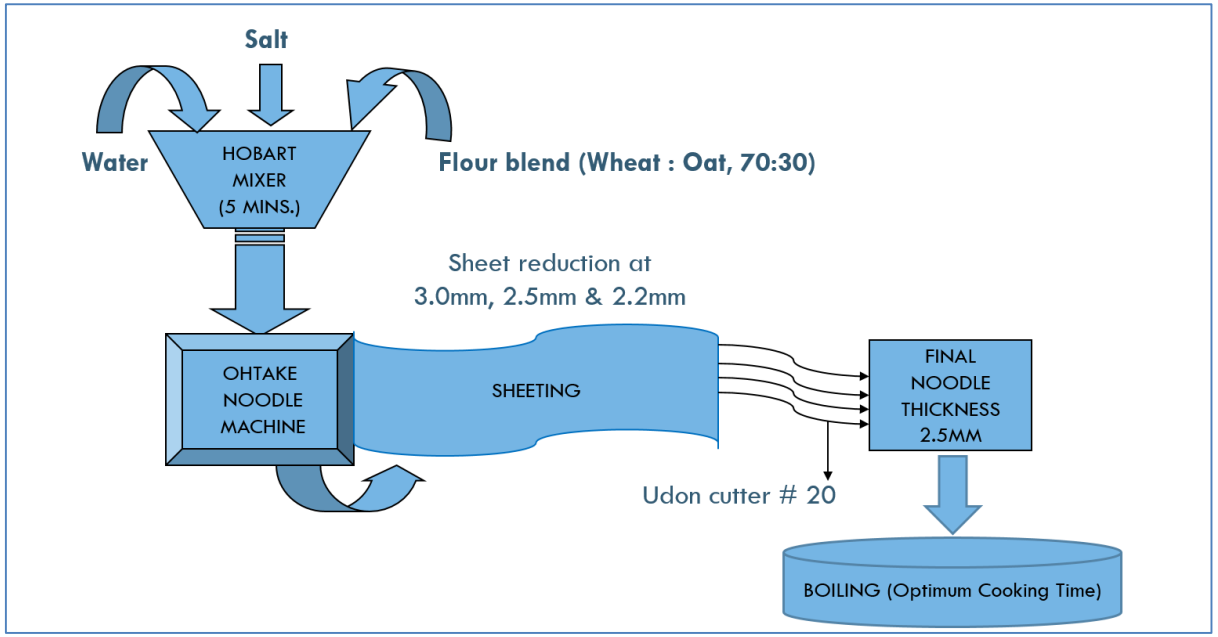

Figure 3.2 Processing of 30% OW noodles

3.4.4.1 *β-glucan content of cooked noodles*

The (1-3) (1-4) - β - D glucan analysis of cooked noodles was conducted using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland). The standard procedure (Megazyme International Ltd., Wicklow, Ireland) used for cooked, toasted or extruded cereal products was followed to analyse the cooked 30% OW noodles and control udon noodles β-glucan content. This assay is based on the principles of approved method 32-23.01 (AACC International 2010) with the procedure explained in section 3.3.2 for the flour samples with some modifications. The cooked samples required a higher (0.2 g) sample size for the assay, pre-extraction with aqueous ethanol to remove free sugars, and 2 mL sodium acetate buffer quantity. Prior to the analysis, the cooked noodles were freeze-dried (Alpha 1-2 LD Plus, John Morris Scientific, Sydney, Australia) with a set protocol of freezing for 30 min; main drying for 20 h at – 30°C (0.37 mbar) and final drying for 30 min at -50°C (0.040 mbar). The freeze dried noodle samples were then ground to a powder for 1 min in a coffee grinder (Breville; Model No. BCG300) set to give fine powder.

3.4.4.2 *Solid loss in cooking water*

The solid loss from cooking the noodle samples (30% OW noodles and control udon noodles) into the cooking water was determined according to AACC method (66-50.01). 25 g (5 cm) of noodles were cooked in 250 mL boiling water as per its optimum cooking time (OCT) as mentioned in section 3.4.3. The cooking water was then collected in a tared beaker and dried to constant weight in an air oven at 105°C for approximately 20 h. The residue obtained in the tared beaker after drying was weighed and the solid loss during cooking was calculated as a percentage of the starting material (25 g).

3.4.4.3 *β-glucan loss in cooking water*

The (1-3) (1-4) - β - D glucan content of cooking water of 30% OW noodles and control udon noodles was conducted using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland). The standard procedure used for wort samples was used for this analysis based on EBC (European Brewery Convention) method 8.13.01. Finely milled ammonium sulphate crystals were dissolved in the cooking

water and were allowed to stand at 4°C for 20 h approximately. The residue after centrifugation was used for the analysis of β-glucan.

3.4.4.4 *Firmness of cooked noodles*

The firmness of cooked noodles was analysed using a texture analyser (TA.XT plus) (Stable Micro Systems, UK) based on Sissons et al (2008) method. The raw noodles were cooked to OCT, drained and placed in a beaker containing 250 mL of distilled water at 25°C for 2 min, and then drained and rested for 1 min before texture analysis was carried out. Noodles were wrapped during the resting time to prevent the noodles strands from drying out. Noodle strands were placed side by side (flatter wider surface side up) on the TA.XTplus base plate of the texture analyser. The strands were cut crosswise with the blade. Three subsequent cuts from the same cook were conducted at an interval of 45 sec.

The texture analyser was equipped with 5 kg load cell and a cutting blade (light knife blade-A/LKB-F). The standard protocol was set (test mode: force of compression, crosshead height calibration: 10 mm, Test speed: 2 mm/sec, post-test speed: 10 mm/sec, distance blade travels: 9.9 mm. trigger: button, unit of force: g and unit distance: mm). Noodle firmness (height of the peak g/force), or maximum cutting force was determined (Sissons et al 2008). Texture profile analysis (TPA) was not conducted on these samples because of the large number of subsequent tests needed and limited sample. This procedure was performed in triplicate for replicate samples.

3.4.4.5 *Colour of noodle sheet and cooked noodles*

Colour analysis of raw noodle sheets and cooked noodles was conducted using a Konica Minolta Chroma Meter (CR-310, Konica Minolta, Tokyo), to measure the CIE L^* (brightness or lightness), a^* (redness and greenness), and b^* (yellowness and blueness) values (Solah et al 2007; Aydin and Gocmen 2011; Mitra et al 2012). CIE L^* of double folded raw noodle sheet was assessed at 0 h and after 24 h (after storing in a Contherm cabinet at 25°C) on a standard white background (backing tile) (Morris et al 2000; Solah et al 2007). The noodles were cooked based on the OCT (optimum cooking time) as mentioned in section 3.4.3. The cooked noodle samples (60 g) were held in a covered plastic jar for 15 min after boiling. The Minolta colour readings were

taken using an Agtron sample cup (311595, Agtron, Reno, NV) to compress the noodles (Solah et al 2007).

3.5 Measurement of β-glucan viscosity

The Gamel et al (2012; 2014) method was used to determine the viscosity of β-glucan using the Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia). Some modifications were made to this method, for both the 100% oat flour and 30% OW noodle samples tested.

3.5.1 Sample preparation

The method developed by Gamel et al (2012; 2014) was based on cooked samples; therefore the oat flour samples in this study were also pre- cooked to study the β-glucan viscosity of extract from raw oat flour. In the modification step the 100% oat flour samples were first cooked in the RVA canisters with the ratio of water to oat flour, and the time of cooking based on Beer et al (1997b) method. Two g (on dry weight basis) of 100% oat flour samples were weighed into a RVA canister. A volume of 12 mL boiling water was added to the flour sample and this mixture was heated in a boiling water bath for 30 sec. The sample was cooled for 10 min and 13 mL of buffer (20 mM sodium phosphate buffer +10 mM NaCl, pH 6.9) was added to the RVA canister. For the noodle samples, control and 30% OW noodles, 4.5 g (on dry weight basis) of freeze dried powder (refer to section 3.4.4.1) was weighed into a RVA canister and 25 mL of buffer (20 mM sodium phosphate buffer +10 mM NaCl, pH 6.9) was added to the RVA canister.

3.5.2 Treatment with enzymes

Gamel et al (2012; 2014) extensively studied various combination and concentration of digestive enzymes. For this study, the best combination of digestive enzymes suggested by Gamel et al (2012; 2014) was applied. Although from Gamel et al (2014) study it was concluded that pancreatin and microbial α-amylase would provide the optimum conditions for enzymatic extraction and viscosity, a concern with the pancreatin was that it may not be consistent from different suppliers, as it is extracted from animal sources. Thus, Gamel et al (2014) suggested microbial protease as the best alternative. A previous study by Gamel et al (2014) did not find any significant change in viscosity with the addition of lipase, thus, this enzyme was not used for this study. Amounts of 20 U of Microbial protease (*Bacillus licheniformis)* (Megazyme E-

BSPRT100; EC.3.4.21.14, 300 U/mL), and 30 U of Microbial amylase *(Bacillus licheniformis)* (Megazyme E-BLAAM100; EC.3.2.1.1, 3000 U/mL) were applied for this study as shown in Figure 3.3 and 3.4.

3.5.3 Method

Pre-cooked oat flour samples and freeze dried 30% OW noodle samples were mixed with the sodium phosphate buffer (pH 6.9) in the RVA canisters and the enzyme combination of microbial α-amylase (30 U) and microbial protease (20 U) was added.

The RVA (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was programmed to run with the following profile: samples were held at 37°C for the duration of the test, and the mixing speed was set at 480 rpm for the first 10 sec followed by 2 h at 160 rpm. The final viscosity of the slurries (mixture of the sample, buffer and enzyme) from oat flour and 30% OW noodle samples was recorded at the end of the test.

After enzyme treatment the sample slurry (mixture of the sample, buffer and enzymes) of oat flour/30% OW noodle was centrifuged at 8000 x g at 20°C for 10 min. The supernatant containing the extracted soluble β-glucan was analysed for viscosity, β-glucan content, β-glucan solubility and β-glucan molecular weight.

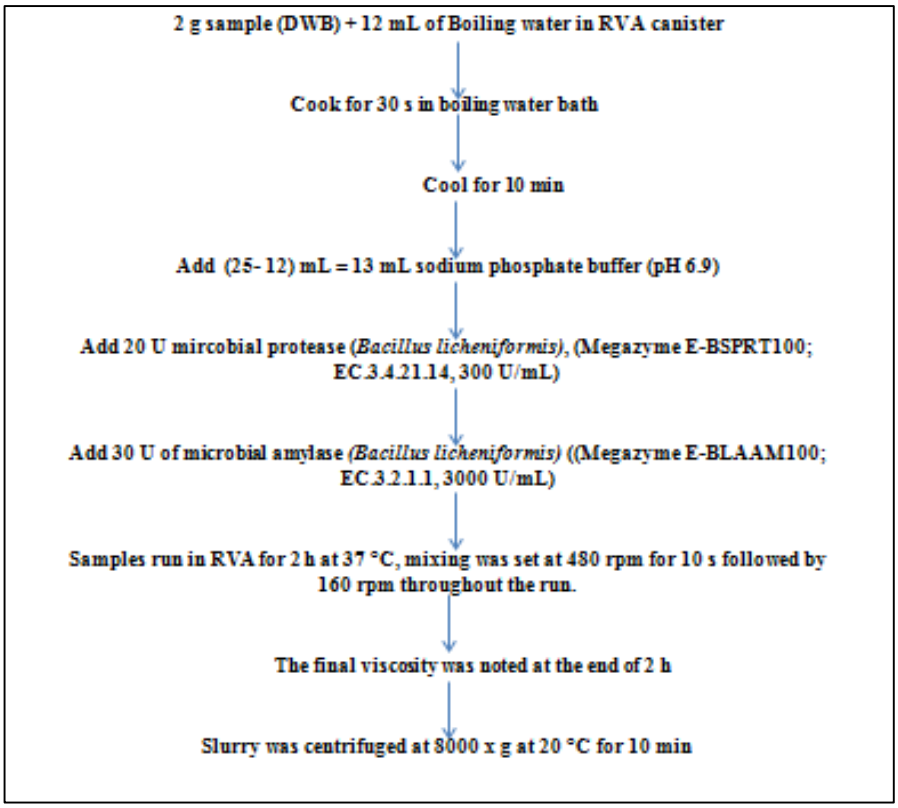

Figure 3.3 Soluble β-glucan extracted from 100% oat flour

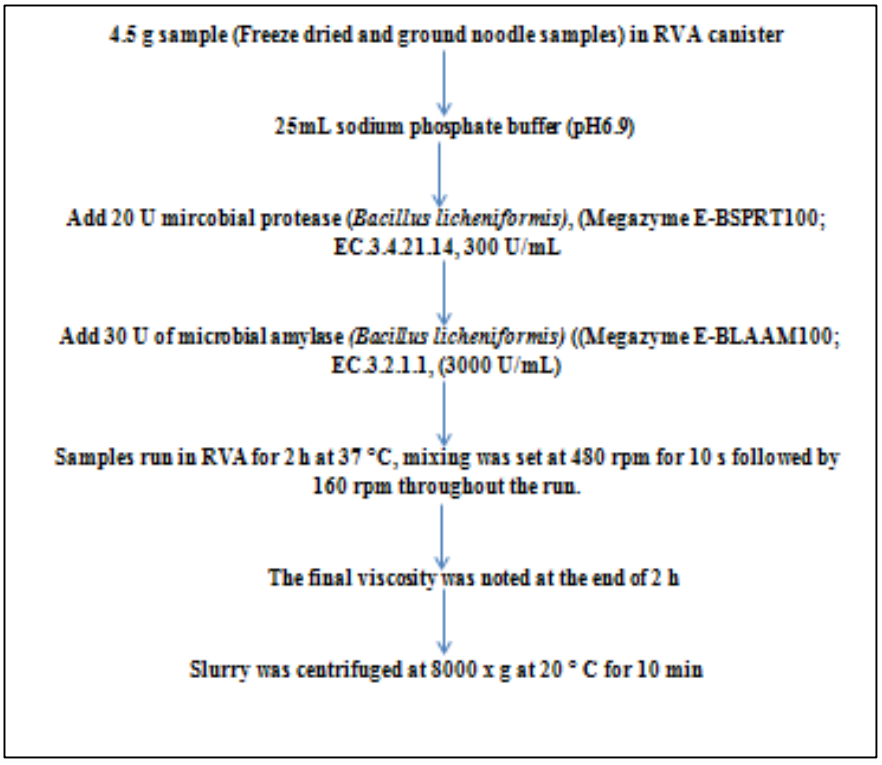

Figure 3.4 Soluble β-glucan extracted from 30% oat-wheat WSN

3.5.3.1 *Extract Viscosity*

The viscosity of the supernatant containing β-glucan was measured using a viscometer (Brookfield, DV-I + viscometer) with enhanced UL adapter kit (Brookfield Engineering Labs. Inc., Stroughton, USA) with ULA spindle (shear rate 122 sec^{-1}) to measure low viscosity materials (Turnbull et al 2005) and a Small Sample Adapter Kit (Brookfield Engineering Labs. Inc., Stroughton, USA) with cylindrical spindle SC4-29 spindle (shear rate 25 sec^{-1}) to measure viscosity of 100% oat flour extract with high viscosity. Both of these adapters had a water jacketed assembly to maintain the temperature of the samples at 37°C. The supernatant viscosity was measured after removing the interference of particulate matter in the solubilised β-glucan extract from RVA and was correlated to the RVA final viscosity of 100% oat flour slurry and 30% OW noodle slurry.

3.5.3.2 *β-glucan content of the extract*

The centrifuged extracts (supernatant) of 100% oat flour and 30% OW noodles containing the soluble β-glucan were frozen at -80°C in a ultralow temperature freezer (Thermo Scientific) and then freeze dried in a freeze dryer (Alpha 1-2 LD Plus, John Morris Scientific, Sydney, Australia) with a set protocol of: freezing for 30 min; main drying for 20 h at – 30°C (0.37 mbar) and final drying for 30 min at -50°C (0.040 mbar).

The β-glucan content of the ground freeze dried supernatants from 100% oat flour and 30% OW noodles was analysed using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland) using the standard procedure for cooked, toasted or extruded cereal products based on approved method 32-23.01 (AACC International 2010) as mentioned in section 3.4.4.1.

3.5.3.3 *Solubility*

The percent extractable (solubilised) β-glucan at 37°C was calculated from the following formulae (Tosh et al 2010):

% soluble β-glucan = (soluble β-glucan/total β-glucan) x100

The soluble β-glucan of 100% oat flour and 30% OW noodles was analysed as mentioned in section 3.5.3.2 and the total β-glucan of 100% oat flour and 30% OW noodles was analysed as mentioned in sections 3.3.2 and 3.4.4.1, respectively.

3.5.3.4 *Molecular weight of β-glucan*

The peak molecular weight (M_p) and average molecular weight (M_a) of the extracted β-glucan from 100% oat flour and 30% OW noodle samples, was measured using high performance size exclusion chromatography (HPSEC) (Agilent 1100/1200 HPLC) with post column addition of fluorescent brightener (FB) 28 (Calcofluor) and fluorescence detection (Shimadzu fluorescence detector RF-10AXL).

The freeze dried supernatants of oat flours and 30% OW noodles were dissolved at a concentration of 10 g/L in 20 mM phosphate buffer, pH 6.8 (0.02% sodium azide) at 100°C with occasional mixing (Wood et al 1991b). The samples were then centrifuged at 208,000 rcf for 5 min. Chromatography was carried out on an Agilent 1200 LC. Samples, 20 µL were injected with an autosampler onto a Polysep-GFC-P6000 (range 100 K - 15 M Da) and Polysep-GFC-P5000 (range 50 K - 2 M Da) (both 300X7.8mm) in series with a guard column. The flow rate was 0.5 mL/min and the columns were maintained at 50°C. Fluorescent brightener 28 (Calcofluor) at 25 mg/L in 0.1M Tris buffer, pH 8, HCl was added post column at 0.5 mL/min and the resultant solution was passed through a 0.25 mm i.d. peak coil 2.5 m long, also maintained at 50°C. A Shimadzu RF-10AXL was used for fluorescence detection, Ex 415nm Em 445nm. Although six (1-3) (1-4)-β glucan standards of known molecular weights obtained commercially (Megazyme standards, 33.6 kDa to 667 kDa) were run through the columns to get the chromatography for all standards, only five standard (67.1 kDa to 667 kDa) values were used to construct a calibration curve (retention time vs. log Mp) because the lower standard 33.6 kDa was outside the range of the two columns (50 KDa – 15 MDa) used for this analysis. The peak molecular weight (Mp) and average molecular weight (Mw) of the extracted β-glucan was calculated using this calibration.

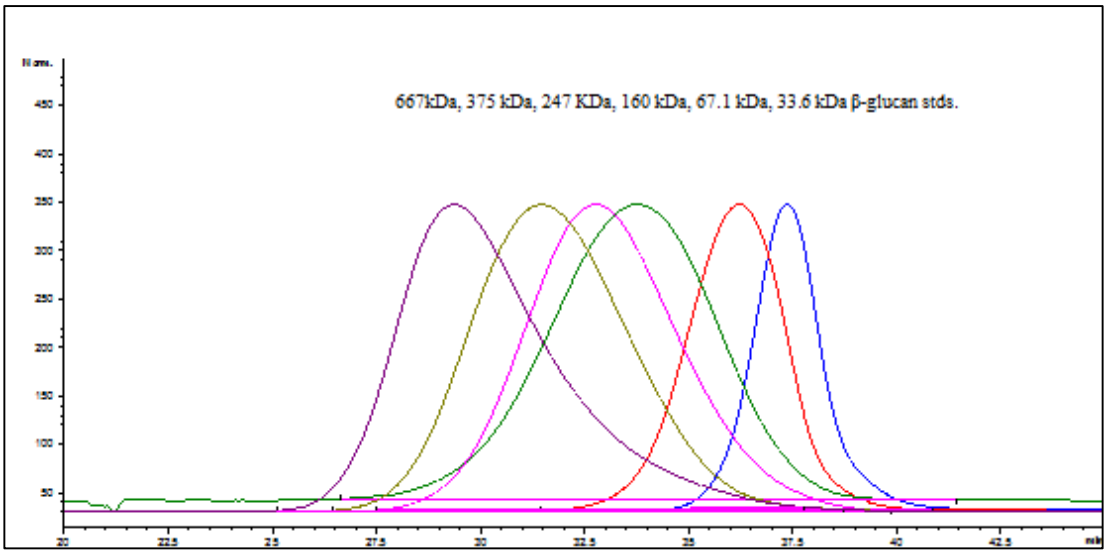

Figure 3.5 Size exclusion chromatograms of 6 β-glucan standards

Overview of Results and Discussion

The following results and discussion section is divided into three studies.

Study 1: Physicochemical properties of oat cultivars (Chapter 4)

Main objective:

- Characterise the flour from different oat cultivars and growing seasons in terms of its physicochemical properties such as pasting properties, β-glucan content and proximate analysis, starch composition, particle size and colour.

Study 2: Oat-wheat noodle quality (Chapter 5)

Main objective:

- Evaluate the effect of incorporating oat flour from different oat cultivars and growing seasons on the quality of white salted noodles (WSN).

Some information from study 1 and study 2 has been accepted for publication as follows:

Mitra, S., James, A.P., Fenton, H.K., Cato, L., and Solah, V.A. 2015. The impact of oat quality on white salted noodles containing oat flour. Cereal Chem.

Study 3: Effects of processing on oat β -glucan and its physicochemical properties (Chapter 6)

Main objectives:

- Determine the physicochemical properties of β-glucan isolated from oat flour and oat enriched white salted noodles, of different oat cultivars and growing seasons.
- Evaluate the effect of processing on the susceptibility of β-glucan to breakdown of different oat cultivars and growing seasons.

Chapter 4: Study 1- Physicochemical Properties of Oat Cultivars

4.1 Introduction

Research has shown that increased consumption of whole grain foods, including oats reduces the risk of metabolic syndrome (Pins et al 2002; Esmaillzadeh et al 2004; Sahyoun et al 2006; Calton et al 2014). The nutritional benefits of whole grain dehulled oats are related to the soluble and insoluble dietary fibre, nutritionally superior protein composition, antioxidant content, fatty acid profile, and content of other micronutrients (Peterson 2001; Webster 2002; Flander et al 2007; Rasane et al 2015). In oat and barley cereal grains, high levels of mixed-linked (1→3) (1→4)-β-D-glucan are found in the endospermic cell wall (Webster 2002; Brennan and Cleary 2005; Wood 2007).

There is growing interest by consumers and researchers in the potential health benefits of β-glucan consumption resulting from the functional characteristics that attenuate the metabolic abnormalities associated with metabolic syndrome and improve cardiovascular disease risk (Smith et al 2008; El Khoury et al 2012). Consumption of oat β-glucan is associated with the inhibition of intestinal cholesterol reabsorption and hence controls circulating cholesterol concentrations (Ripsin et al 1992; Davy et al 2002; Wood 2007; Tiwari and Cummins 2011). Meta-analyses of clinical studies showed a reduction of total cholesterol by 0.13 mmol/L with an intake of 3 g of soluble fibre from oats (Ripsin et al 1992; Brown et al 1999). A recent meta-analysis by Whitehead et al (2014) showed a reduction in LDL cholesterol by 0.25 mmol/L and total cholesterol by 0.30 mmol/L with an intake of ≥ 3 g/d of β-glucan from oats. The European Union health claims for barley and oat β-glucans are related to the maintenance and reduction in the blood cholesterol with consumption of 3 g of β-glucan from oat and barley and reduction in postprandial glycaemia with the consumption of 4 g of β-glucan/30 g available carbohydrate (Harland 2014). The positive effect of β-glucan on postprandial blood glucose and insulin levels has been reported (Jenkins et al 2002; Biorklund et al 2005; Dongowski et al 2005). In a randomised cross-over study on men and women with type 2 diabetes, each g of β-glucan reduced the glycaemic index (GI) by 4 units after consumption of a 50 g carbohydrate portion of oat incorporated breakfast cereals (Jenkins et al 2002).

Australia has gained a reputation in the world for growing high quality milling oats (premium quality oats, used for human consumption) for local and export markets (DAFWA 2006). The total production of oats in Western Australia (WA) averaged 506 kt over the period 2009-2013 (ABARES 2015). Oats produced in Western Australia have a good reputation internationally because of their bright colour, plump grain, low levels of admixture or contamination, high groats level, high milling yield, and are less prone to frost damage and water logging (DAFWA 2006; 2015).

The genetic and environmental factors have been reported to impact on a number of important compositional, functional and sensory characteristics of oats. Genotype and environment affect the nutritional composition of the grains (β-glucan, protein and lipid), and therefore, health benefits, as well as agronomic characteristics (Doehlert et al 2001; Peterson et al 2005). Grain hardness and β-glucan content and also environment can impact on a range of milling properties such as groat breakage, milling yield and particle size distribution (Doehlert and McMullen 2000; Hüttner et al 2010). β-glucan in the cell wall is known to confer toughness and reduce damage during milling (Engleson and Fulcher 2002). Bran flake contamination during milling, particle size distribution and inherent endosperm colour of grains have an impact on flour colour (Mares et al 2008; Ram and Mishra 2010; Wang et al 2004; Salehifar and Shahedi et al 2007). Characteristics of oat flour resulting from the milling properties, β-glucan content, molecular structure of β-glucan and its interaction with other oat components influence the viscosity of oat slurries (Becker et al 2001; Liu et al 2010; Choi et al 2012). The physicochemical properties of oat flour; pasting properties, starch swelling properties and gelatinisation are important in the processing of all end products undergoing heat treatment in the presence of water (Rhymer et al 2005). The impact of genotype and environment on oat grain functionality and composition influences all processes from milling to end products.

However, more research is required to understand the impact of environment and genotype on physicochemical properties of oats which can affect end product quality. The objective of this study was to characterise the physicochemical properties of oat flour from different oat cultivars harvested from two growing seasons.

4.2 Summary of materials and methods

4.2.1 Materials

Six milling oat cultivars of Western Australia (Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams) grown over two different seasons (2011 and 2012) at the same location (Katanning, WA) were selected for this study. Oat cultivars were supplied by the National Oat Breeding Program managed by South Australian Research and Development Institute (SARDI), in collaboration with the Department of Agriculture and Food Western Australia (DAFWA). All tests were performed in at least duplicate. Some of the oat flour quality parameters were compared to a wheat flour (control) sample, which was standard commercial white salted noodle (udon) flour from Japan.

4.2.2 Sample preparation

Oat grains were milled into flour (Refer to *section 3.2. Sample preparation* for further details)

4.2.3 Flour analysis

Wheat flour and oat flour total protein content was determined by the Dumas combustion method using a protein/nitrogen analyser (Leco Elemental rapid N cube), according to the approved method 46-30.01 (AACC International 2010). Ash analysis was according to AACCI (2010) approved method 08-01.01. Lipid content in oat groats were determined by NMR (Newport; Oxford UK), calibrated with vegetable oil and in control wheat flour was analysed by approved method 30-10.01 (AACC International 2010). (Refer to *section 3.3.Oat groat and oat flour characterisation* for further details)

The particle size distribution of the wheat flour and oat flour samples were analysed using a laser light scattering using a Mastersizer 2000 (Malvern instruments Ltd, Malvern, UK) based on approved method 55-40.01 (AACC International 2010) (Refer to *section 3.3.Oat groat and oat flour characterisation* for further details)

The amylose content of starch (% w/w) in oat flour was determined using the Megazyme method (Megazyme International Ltd., Wicklow, Ireland) (Gibson et al 1997). Starch damage of the oat flour samples were analysed according to the approved methods (76-31.01, AACCI 2010; Gibson et al 1992), using the starch damage assay kit (Megazyme International Ltd., Wicklow, Ireland). The (1-3)(1-4)-β-D glucan in the control wheat flour, oat flour and was analysed using a mixed-linkage β-glucan kit assay (Megazyme International Ltd., Wicklow, Ireland) using approved method 32-

23.01 (AACC International 2010). (Refer to *section 3.3.Oat groat and oat flour characterisation* for further details)

The CIE *L**, *a** and *b** of oat groats was measured using samples glass light projection tube attachment (CR-A33e). The oat flour and oat flour fractions (>100 μm and <100 μm) CIE *L**, *a** and *b** colour was measured using a Minolta Chroma Meter (Model CR-400, Konica Minolta) with the standard narrow light projection tube attachment and flour analysis attachment cell (TC 189-0564F; Technicon Instruments Corporation). (Refer to *section 3.3.Oat groat and oat flour characterisation* for further details)

4.2.4 Pasting properties

A Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used to determine the pasting properties of oat flour with 20 min pasting profile (Zhou et al 1999) (Refer to *section 3.3.Oat groat and oat flour characterisation* for further details).

4.3 Statistical analysis

Data was analysed using SPSS Version 21 (SPSS, Chicago, IL). The effects of cultivars, years and the interaction between cultivar by years were assessed using analysis of variance (ANOVA) with before and after adjustment for covariates as appropriate. Comparisons of means for significant effects within the ANOVA were carried out using Least Significant Difference (LSD). Pearson's correlation analysis was applied to find correlations between different variables (oat flour composition with RVA viscosity parameters; oat flour particle size or color of groat with oat flour colour). Pearson's correlation coefficients which were strong (r = 0.7 - 0.9) and moderate (r = 0.4 - 0.6) were included in this study. P<0.05 was considered as statistically significant.

4.4 Results and discussion

4.4.1 Oat flour quality

The protein content, ash content, β-glucan content, lipid content and particle size of the oat samples were significantly higher (P<0.05) than the control wheat flour (protein content, 10.0%; ash content, 0.37%; β-glucan content, 0.36%; and lipid content,0.65%). Milled wheat flour particles were mostly <100 μm (78.8%) and >100

μm (16.8%). The main effect of oat cultivars (genotype) (mean of a cultivar over 2 years) was significant (P<0.05) for protein content, ash content, lipid content, amylose, β-glucan content, level of starch damage and particle size.

The main effect of the year of cultivation (environment) (mean across all cultivars within each year) was significant (P<0.05) for protein content, lipid content, ash content, starch damage, and particle size (>100 μm and <100 μm); and not significant (P>0.05) for β-glucan and amylose content. The mean across all cultivars grown in 2012 was significantly (P<0.05) higher for protein content, lipid content, and had greater volume of finer particle size (< 100 μm); and was lower for ash content, starch damage and had lower volume of coarser particle size (>100 μm), in comparison to the mean across the same cultivars grown in 2011. The year of cultivation (environment) by cultivar (genotype) interaction was significant (P<0.05) for ash content, protein content, β-glucan content, starch damage and particle size (>100 μm and <100 μm); and was not significant (P>0.05) for amylose and lipid content.

Protein content of oat cultivars ranged from 13.7 - 18.6% and was within the reported range (11.0 - 20.0%) by Webster (2002) and highest for Carrolup (18.6%) in 2012 and Mitika in 2011 (18.0%) and 2012 (18.3%) (Table 4.1). The ash content ranged from 1.01 -1.34%. Ash content of Mitika (1.34% in 2011; 1.29% in 2012) and Kojonup (1.34% in 2011; 1.23% in 2012) was significantly higher (P<0.05) than the other oat cultivars for both the years (Table 4.1). The lipid content of the oat cultivars (5.5 - 9.1%) was within the reported range (Hareland and Manthey 2003). The lipid content (average over two years) (refer to *Appendix 5* for further details) of Bannister (8.9%) was significantly higher (P<0.05) and Yallara (5.6%) significantly lower (P<0.5) compared to other oat cultivars.

Table 4.I Results for oat flour cultivars from the 2011 and 2012 growing seasons

Cultivars	Protein^ % (dwb)		Ash^ %		[x]Lipids # %		β-glucan ^ % (dwb)		Amylose # %		Starch Damage^ %		Particle Size^ % (in vol.) >100 µm		Particle Size ^ % (in vol.) <100 µm	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	18.0a ±0.04	18.3a ±0.13	1.34a ±0.014	1.29a* ±0.01	6.9 ±0.04	7.7 ±0.08	5.38b ±0.08	4.93c* ±0.11	19.9 ±0.14	19.7 ±0.39	1.83c ±0.03	1.46bc* ±0.01	44.6c ±1.04	37.5d* ±0.17	54.4b ±1.03	61.6a* ±0.20
Kojonup	16.7c ±0.04	17.6b* ±0.25	1.34a ±0.01	1.23b* ±0.01	5.8 ±0.41	6.6 ±0.16	4.90c ±0	5.31b* ±0.01	21.1 ±0.07	22.0 ±0.46	1.84c ±0.014	1.64a* ±0.03	45.3c ±0.13	41.7c* ±0.37	53.4b ±0.21	56.7b* ±0.21
Yallara	16.2d ±0.08	16.1d ±0.16	1.18b ±0.01	1.06d* ±0.014	5.5 ±0.01	5.8 ±0.06	4.59e ±0.02	4.56e ±0.04	21.6 ±0.64	20.5 ±0.18	2.14a ±0.04	1.29d* ±0.01	44.0cd ±1.22	42.4c* ±1.15	54.8ab ±1.14	55.9b ±0.60
Bannister	13.7f ±0.01	15.6e* ±0.01	1.14c ±0.014	1.01e* ±0.014	8.7 ±0.18	9.1 ±0.21	4.76d ±0.08	4.79cd ±0.04	22.6 ±0.11	22.2 ±0.5	1.93b ±0.01	1.44c* ±0.02	51.2b ±0.76	44.6b* ±0.38	48.2c ±0.77	54.2c* ±1.24
Carrolup	17.5b ±0.01	18.6a* ±0.23	1.19b ±0.028	1.11c* ±0.02	6.1 ±0.06	6.8 ±0.02	5.00c ±0.14	4.74d* ±0.03	20.6 ±0.21	20.5 ±0.07	1.71d ±0.03	1.50b* ±0.04	42.8d ±0.92	41.6c ±0.27	56.3a ±0.79	57.4b ±0.33
Williams	15.1e ±0.27	17.3c* ±0.15	1.12c ±0	1.05d* ±0	8.2 ±0.23	8.5 ±0.53	5.92a ±0.01	5.93a ±0	21.1 ±1	22.0 ±1.1	1.72d ±0.01	1.28d* ±0.01	58.2a ±0.16	49.4a* ±0.16	41.3d ±0.14	50.0d* ±0.14

^ Year and cultivar interaction was significant ($P<0.05$); # Year and cultivar interaction was not significant ($P>0.05$); a Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$); [x]Oat groat value was considered for lipids;

The β-glucan content of the oat cultivars grown over two years (4.56 - 5.93%) was within the range reported by others (Colleoni-Sirghie et al 2003; Zwer et al 2013). The β-glucan content of oat cultivars was highest for Williams (5.92% in 2011; 5.93% in 2012) and lowest for Yallara (4.59% in 2011; 4.56% in 2012) (Table 4.1). The amylose content of the oat flours was 19.7-22.6% (Table 4.1), being within the range previously reported by Zhou et al (1998b). The main effect of cultivar (average over two years) on amylose level (refer to *Appendix 5* for further details) showed a significantly higher ($P<0.05$) value for Bannister (22.4%) and a lower value for Mitika (19.8%) in comparison to the other oat cultivars. The starch damage of oat flour in the present study ranged from 1.28 - 2.14% which was lower than the range stated by Tester and Karkalas (1996) of German oat cultivars which ranged from 1.9 - 2.2% (Table 4.1).

The particle size distribution curve from the oat flour analysis for all cultivars shows a bimodal distribution (Figure 4.1 and 4.2) for both the years. Wheat flour particle size distribution curve has previously been reported as a singular mode distribution by Sabaniz and Tzia (2009), although bimodal distribution for wheat flour is reported by Hareland (1994). In this study, the differences between mode of distribution between wheat flour, oat flours and the cultivar and seasonal impact amongst the oat cultivars can be attributed to the differences in their composition as reported in Table 4.1. The oat flour divided into two modes: above 100 μm (coarse/large) and below 100 μm (fine/small). The finer particle size of oat flour mainly would consist of the starchy endosperm (Foehse 1991). The coarser particles would be mostly larger particle starch adhering to the sub aleurone layer and cell wall constituents (Foehse 1991) along with bran as whole groat flour was used in this study. The cell wall constituents and bran could be more resistant to milling thus producing coarser particles.

Particle size distribution analysis (Malvern instruments Ltd, Malvern, UK) showed, that the percentage of coarser particle size (>100 μm) was in the range of 42.8 - 58.2% in 2011 and 37.5 - 49.4% in 2012. The percentage of finer particles (<100 μm) was in the range of 41.3 - 56.3 % in 2011 and 50.0 - 61.6 % in 2012. Williams had the highest percentage of coarser particles (>100μm) and lowest percentage of finer particles (<100 μm) for both years (Table 4.1).

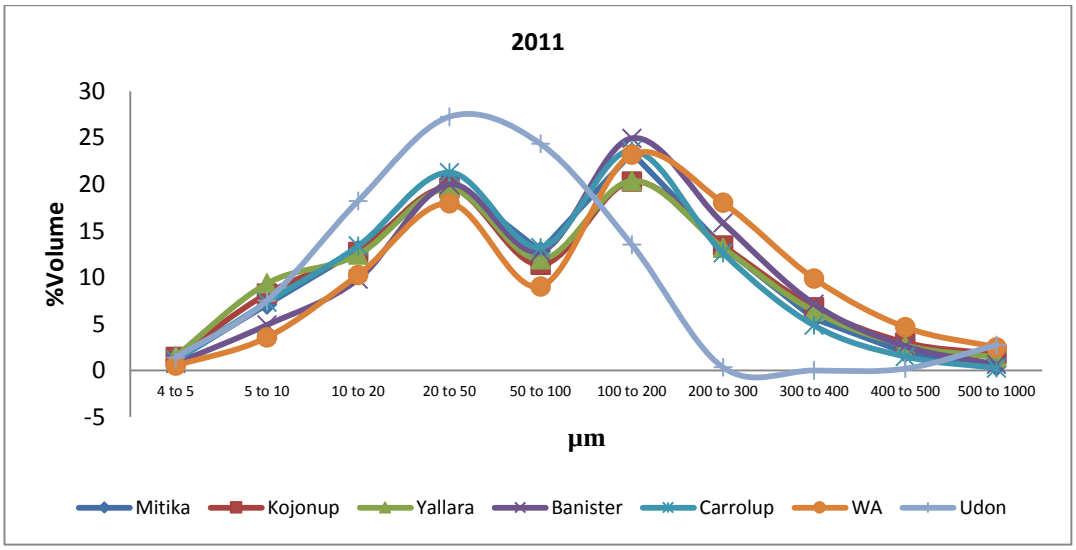

Figure 4.1 Bimodal distribution of particle size of six different oat cultivars for 2011 and control (udon) wheat flour

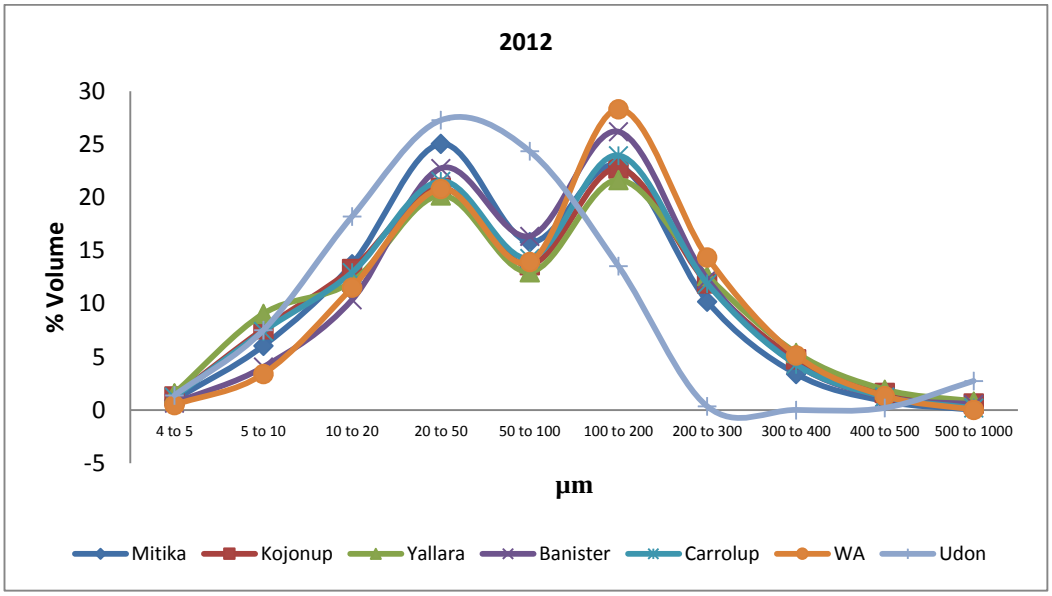

Figure 4.2 Bimodal distribution of particle size of six different oat cultivars for 2012 and control (udon) wheat flour

4.4.2 CIE (*L*a* b**) colour values of oat groat, oat flour and oat fraction

Australian oat grains have an excellent reputation for their high quality (DAFWA 2015). In addition to plumpness of grain, brightness is also an important aesthetic feature of Australian oats which is preferred by end users (DAFWA 2015). Colour quality of oat grain, oat flour and flour fraction is discussed in the following section

Table 4.2 CIE (*L*a*b) colour values of oat grain**

Cultivars	L*^		a*^		b*^	
Oat grain	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	55.85b ±0.06	58.17b * ±0.02	7.89c ± 0.03	8.93b* ± 0.02	19.99c ± 0.26	21.46c* ± 0.02
Kojonup	54.67c ±0.43	57.14cd* ± 0.19	8.58a ± 0.03	9.47a* ± 0.01	19.80c ± 0.09	20.94d* ± 0.06
Yallara	56.47a ± 0.05	61.93a * ± 0.02	7.66d ± 0.04	7.48d* ± 0.14	21.62a ± 0.18	22.49a* ± 0.02
Bannister	53.04d ± 0.04	58.14b* ± 0.06	7.67d ± 0.07	8.76c* ± 0.28	19.08d ± 0.11	22.04b* ± 0.14
Carrolup	55.02c ± 0.02	57.44c* ± 0.56	8.32b ± 0.04	9.51a* ± 0.01	20.91b ± 0.17	21.06d ± 0.38
Williams	52.98d ± 0.04	56.70d * ± 0.09	7.06e ± 0.04	7.54d* ± 0.06	18.52e ± 0.03	19.40e* ± 0.12

^ Year and cultivar interaction was significant ($P<0.05$); [a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$).

For the colour of oat groats (Table 4.2), CIE *L**(brightness) and *b** (yellowness) values were highest for Yallara (*L**: 56.47 for 2011 and 61.93 for 2012; *b**: 21.62 for 2011 and 22.49 for 2012) and CIE *a* (*redness) value was highest for Kojonup (8.58 for 2011 and 9.47 for 2012) cultivar for both the years. CIE *L* (*brightness) and . CIE *b** (yellowness) values were lowest for Williams (*L**: 52.98 for 2011 and 56.70 for 2012; *b**: 18.52 for 2011 and 19.4 for 2012) for both the years. The colour of the groats gives the colour of the oat bran. The brightness (CIE *L**) of the oat groats in this study was within the kernel brightness range mentioned by AEGIC (2014b). The colour of oat bran along with the colour of the oat endosperm would affect the colour of the whole oat flour which is given in Table 4.3. (Refer to *Appendix 8* for photographs)

Table 4.3 CIE (*L*a*b) colour values of oat flour**

Cultivars	L*^		a*^		b*^	
Oat Flour	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	85.55b ± 0.08	86.12b* ±0.89	1.25c ±0.013	1.22d ± 0.024	10.29b ± 0.13	10.7b* ± 0.11
Kojonup	85.22b ±0.23	86.41b* ± 0.38	1.53a ± 0.061	1.57a ± 0.023	9.42c ± 0.25	10.1c* ± 0.03
Yallara	86.68a ± 0.35	88.02a* ± 0.2	1d ± 0.004	0.84e* ± 0.042	9.13c ± 0.07	9.31d ± 0.46
Bannister	82.12d ± 0.22	84.37c* ± 0.13	1.55a ± 0.021	1.44b* ± 0.046	10.79a ± 0.02	12.22a* ± 0.15
Carrolup	85.32b ± 0.19	86.30b* ± 0.01	1.33b ± 0.062	1.18d* ± 0.042	10.23b ± 0.1	10.68b* ± 0.16
Williams	83.15c ± 0.09	84.25c* ± 0.29	1.49a ± 0.025	1.34c* ± 0.031	10.67a ± 0.01	11.04b ± 0.01

^ Year and cultivar interaction was significant (P<0.05); [a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05);*Notes the significant difference in mean values between different years (P<0.05)

Flour colour depends on the colour of the bran and also the inherent colour of the endosperm (Mares et al 2008). For the oat flour colour (Table 4.3), Yallara had highest brightness (CIE *L** 86.68 for 2011 and 88.02 for 2012) values and lowest in redness (CIE *a**1.00 for 2011 and 0.84 for 2012) and yellowness (CIE *b** 9.13 for 2011 and 9.31 for 2012) values in comparison to the other oat cultivars. CIE *L** (brightness) value was lowest for Bannister (82.12 for 2011 and 84.37 for 2012) for both the years and Williams (84.25) for 2012. CIE *b** (yellowness) value was highest for Bannister (10.79 for 2011 and 12.22 for 2012) for both the years and redness (CIE *a*)* was highest for Bannister (1.55) in 2011 and Kojonup (1.57) in 2012.

The main effect of year of cultivation, cultivar and interaction between the year of cultivation and cultivar for CIE (*L*a*b**) colour values of oat groat and oat flour was significant (P<0.05). For both oat groat and oat flour samples, Yallara had the highest brightness value and Kojonup was highest in redness value for both years. Williams had the lowest brightness value for both years in comparison to other oat cultivars with the exception of the 2011 William oat flour (which was second lowest in brightness to Bannister 2011). For oat grain and oat flour CIE *L** and *b** colour values, 2012 was higher than the same cultivar grown in 2011 (Refer to *Appendix 8* for photographs).

This could be due to the fact that the total rainfall in 2011 (668.4 mm) was much higher than 2012 (481.0 mm) in Western Australia (Katanning) (Bureau of Meteorology 2013) (Refer to *Appendix 1* for further details) as high temperature and humidity during harvest and grain filling activates peroxidase enzyme and also facilitate mould growth which can lead to discoloration of grains (Marano et al 2012; AWB 2014).

Oat bran had higher amount of red and yellow pigment in comparison to the endosperm (Tian et al 2010), thus, the CIE a^* and b^* values of oat groats were higher than the oat flour. However, since the whole oat flour was used for this study, the flour colour would have been influenced by both the endosperm and bran colour. A strong positive correlation (r = 0.956, P<0.05 for 2011 and r = 0.718, P>0.05 for 2012) was observed between CIE L^* value of oat bran and L^* value of oat flour. A positive correlation was also observed between CIE L^* value of oat flour with the percent volume of finer particles in oat flour (<100 μm) and (0.776, P>0.05 for 2011; 0.542, P>0.05 for 2012). Similar observations were noted by Wang et al (2004) in his study on wheat flour, where granularity affected the flour brightness and finer particle size flour was brighter. Among the flour colour characteristics CIE L^* value of oat flour was negatively correlated to its CIE a^* (r = -0.796 for 2011, P>0.05; r =-0.660 for 2012 P>0.05) and CIE b^* values (r =-0.828 for 2011, P<0. 05; r =-0.894 for 2012, P<0.05) and similar observation was noted by Wang et al (2004) for wheat flour samples.

CIE ($L^*a^*b^*$) colour values were noted for these separated fractions as denoted in Table 4.4 and 4.5. The main effect of year of cultivation, cultivar and interaction between the year of cultivation and cultivar for CIE ($L^*a^*b^*$) colour values of oat flour fractions (>100 μm and <100 μm) was significant (P<0.05).

Table 4.4 CIE (*L*a*b**) colour values of oat flour fraction >100 µm

Cultivars	L*^		a*^		b*^	
	2011	2012	2011	2012	2011	2012
Mitika	82.22b ±0.27	82.70c * ±0.13	1.97d ±0.03	2.01b ± 0.04	13.17ab ± 0.05	13.77b* ± 0.24
Kojonup	79.85d ±0.48	83.10b * ± 0.01	2.38a ± 0.02	2.17a* ± 0.06	12.48c ± 0.09	12.26d ± 0.16
Yallara	82.88a ± 0.42	86.16a * ± 0.001	1.66e ± 0.06	1.17e* ± 0.02	11.68d ± 0.03	11.01e* ± 0.04
Bannister	79.09d ±0.18	81.94d* ± 0.05	2.20b ± 0.09	1.92c* ± 0.02	13.00b ± 0.16	14.36a* ± 0.02
Carrolup	81.06c ± 0.31	82.79bc* ± 0.02	2.11c ± 0.03	1.81d * ± 0.03	13.35a ± 0.12	13.51bc ± 0.16
Williams	79.03e ± 0.21	81.38e* ± 0.18	2.06c ± 0.02	1.87cd* ± 0.04	12.89b ± 0.24	13.22c* ± 0.02

^ Year and cultivar interaction was significant ($P<0.05$); [a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$).

Table 4.5 CIE (*L*a*b**) colour values of oat flour fraction < 100µm

Cultivars	L*^		a*^		b*^	
	2011	2012	2011	2012	2011	2012
Mitika	87.49b ±0.42	87.83b ±0.18	0.87c ±0.01	0.87c ± 0.06	8.88c ± 0.14	8.85c ± 0.02
Kojonup	87.42b ±0.33	87.79b ± 0.22	1.30a ± 0.001	1.26a ± 0.01	8.15d ± 0.003	8.74c* ± 0.002
Yallara	88.43a ± 0.02	89.54a * ± 0.21	0.81d ± 0.01	0.59d* ± 0.01	8.12d ± 0.06	8.13d ± 0.07
Bannister	85.24c ± 0.97	86.51c * ± 0.17	1.25a ± 0.06	0.96b* ± 0.01	9.64a ± 0.31	10.09a* ± 0.05
Carrolup	87.25b ± 0.06	86.79c ± 0.01	1.14b ± 0.00	0.83c* ± 0.004	9.27b ± 0.03	9.25b ± 0.00
Williams	84.51d ± 0.01	85.63d* ± 0.26	1.19b ± 0.01	1.01b* ± 0.01	9.33b ± 0.01	9.23b ± 0.09

^ Year and cultivar interaction was significant ($P<0.05$); [a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$).

For oat flour fraction >100 μm and <100 μm, Yallara was highest in brightness (CIE *L**) (>100 μm *L**: 82.88 for 2011; 86.16 for 2012 and <100 μm*L**: 88.43 for 2011 ; 89.54 for 2012) and lowest in redness (CIE *a**) (>100 μm *a**:1.66 for 2011; 1.17 for 2012 and <100 μm *a**: 0.81 for 2011; 0.59 for 2012) and yellowness (CIE *b**) (>100μm *b**: 11.68 for 2011; 11.01 for 2012 and <100 μm *b**: 8.12 for 2011 and 8.13 for 2012) values in comparison to other oat cultivars for both the years. Williams on the other hand, had the lowest brightness (CIE *L**) (>100 μm *L**: 79.03 for 2011; 81.38 for 2012 and <100 μm *L**: 84.51 for 2011 and 85.63 for 2012) values and Kojonup had the highest redness (CIE *a**) (>100 μm *a**: 2.38 for 2011; 2.17 for 2012 and <100 μm *a**: 1.30 for 2011; 1.26 for 2012) values for both the years in comparison to other oat cultivars.

The oat flour fraction > 100 μm were lower in *L** and higher in *a** and *b** values in comparison to the oat flour fraction <100 μm. For the oat flour fraction > 100 μm, it was visually noted to have brans and coarser flour particles. The oat flour fraction < 100 μm had very fine flour particles without any specks or bran particles. The *L** value of oat flour fraction <100 μm was higher because finer particles size have higher brightness (Wang et al 2004) and also lower bran fractions which has an inherent colour which is darker than the endosperm and have higher red and yellow pigments (Tian et al 2010). According to Hidalgo et al 2014, flour colour is not only determined by flour pigments such as carotenoid content, but also by flour particle size. The highest percentage of coarser particle (Table 4.1) and darker colour oat bran (Table 4.2) may have resulted in Williams oat flour having low brightness value in comparison to other oat cultivars.

4.4.3 Pasting properties

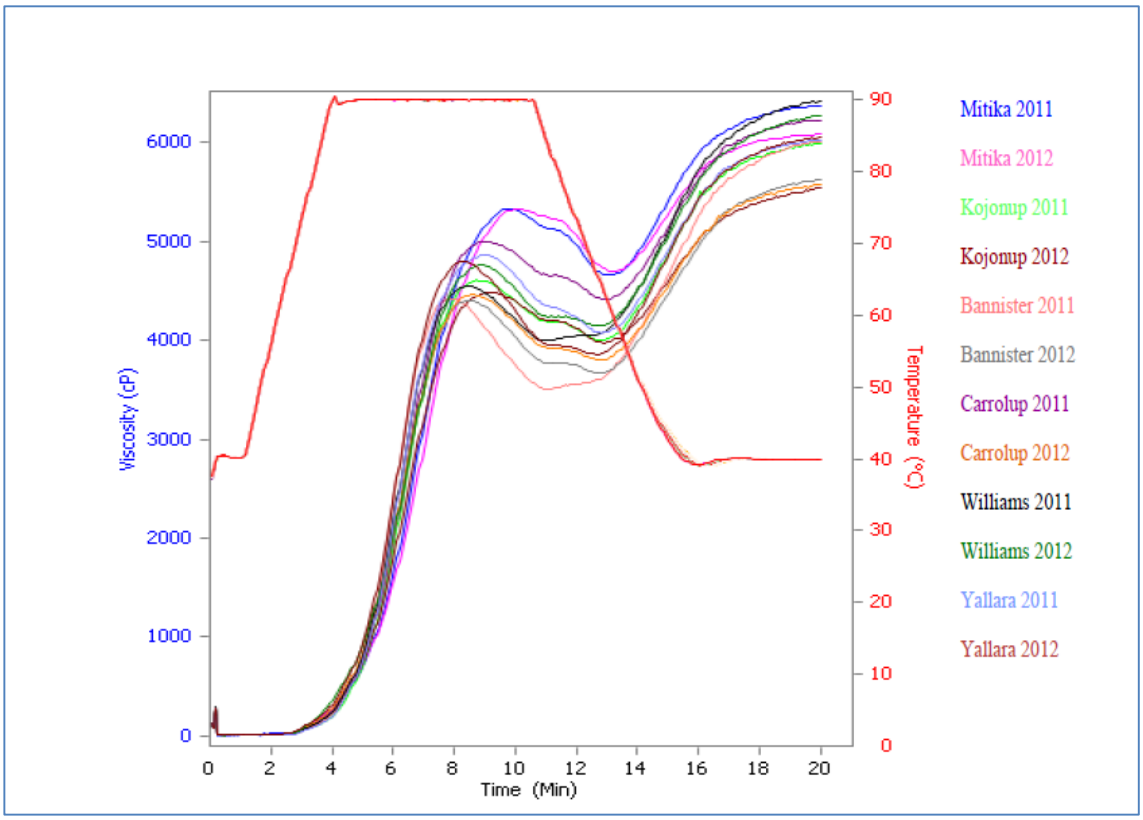

Figure 4.3 RVA pasting properties of 100% oat flour

The RVA viscograph of 100% oat flour shows a relatively small peak viscosity, a small drop in viscosity on cooling and gives a high setback value, indicating the formation of a strong gel and this was similar to the results reported by other researchers (Zhou et al 1998b; Bason and Blakeney 2007; Mitra et al 2012). The pasting curve properties (Figure 4.3, Table 4.6) illustrate the difference in the pasting properties of different oat cultivars over different growing seasons. The RVA curves of 100% oat flour showed a possible double peak, which may be due to the formation of starch-lipid complexes (Nelles et al 2000).

Flour samples containing lipids, β-glucan, protein and amylose (Zhou et al 2000; Zhou et al 1999; Hüttner 2011), together with particle size differences influence the pasting properties. Zhou et al (1998b) states that it is difficult to separate the effect of starch, β-glucan and other components, unless the starch is isolated and its pasting properties studied separately. However, it is unviable to isolate starch from each sample before

testing thus viscosity tests are usually applied on flour samples (Ram and Mishra 2010).

The main effect of year, cultivars and the interaction between year and cultivar were significant ($P<0.05$) for all RVA parameters of oat flour. The peak viscosity and trough viscosity obtained for the oat cultivar Mitika was significantly higher ($P<0.05$) than other oat cultivars for both years (Table 4.6; peak viscosity: 5362 cP in 2011 and 5327 cP in 2012; trough viscosity: 4706 cP in 2011 and 4691 cP in 2012). Even after adjusting for the covariate protein, Mitika still exhibited the highest peak viscosity for both years which is associated with higher swelling of starch granules during gelatinisation (Choi et al 2012).

Mitika had the lowest amylose percentage (19.9% in 2011; 19.7% in 2012), therefore had the highest amylopectin, the highest protein content (18.0% in 2011 and 18.3% in 2012), and the highest percentage of finer particles (54.4% in 2011 and 61.6% in 2012) compared to all other cultivars tested. It is likely that a greater proportion of amylopectin contributed to swelling of starch granules (Tester and Morrison 1990). A strong negative correlation was noted between peak viscosity and amylose percentage for both years ($r = - 0.818$, $P<0.05$ for 2011 and $r = -0.712$, $P>0.05$ for 2012). A study on oat flour slurry viscosity by Zhang et al (1997) noted that oat flour with fine particles has a greater surface area for faster hydration than oat flour with coarser particles. Peak viscosity had a moderate positive correlation with fine particles (<100µm) for both years ($r = 0.623$, $P>0.05$ for 2011; $r = 0.500$, $P>0.05$ for 2012). Moss (1967) previously noted a positive relationship between protein content and peak viscosity. A positive relationship between protein content and peak viscosity was found in our research but the correlation was stronger and significant for the year 2011 ($r = 0.854$, $P<0.05$) compared to 2012 ($r =0.400$, $P>0.05$).

Table 4.6 RVA pasting properties of 100% oat flour

Cultivars	Peak^ (cP)		Trough^ (cP)		Breakdown^ (cP)		Final Viscosity^ (cP)		Setback^ (cP)		Peak Time^ (Min.)		Pasting Temperature^ (°C)	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	5362a ±41.72	5327a ±0.71	4706a ±65.05	4691a ±9.19	656c ±23.33	637c ±9.9	6478a ±160.5	6067b* ±17.67	1772d ±95.46	1376e* ±8.49	9.87a ±0.09	10.04a ±0.05	86.7a ±1.70	82.3b* ±4.67
Kojonup	4530d ±111	4465cd ±21.92	3956c ±65.05	3959c ±21.21	574d ±45.96	506d* ±0.71	5935c ±75.66	5498c* ±56.57	1979c ±10.61	1539d* ±35.36	8.80c ±0.10	9.24b* ±0.05	87.9a ±0.07	87.8a ±0.11
Yallara	4838c ±38.89	4740b ±77.78	4038c ±49.50	3847d* ±17.68	800b ±10.61	894a* ±60.10	6009c ±19.80	6015b ±43.84	1971c ±29.70	2169a* ±26.16	8.90bc ±0.04	8.27e* ±0.00	87.9a ±0.11	85.0ab* ±0.81
Bannister	4369e ±77.78	4385d ±22.63	3478d ±41.72	3648e* ±26.87	892a ±36.06	737b* ±4.24	5960c ±65.05	5640c* ±21.92	2483a ±23.33	1992b* ±48.79	7.90e ±0.04	8.47d* ±0.00	87.9a ±0.11	86.2a ±0.85
Carrolup	4984b ±20.51	4501c* ±55.86	4410b ±3.54	3836d* ±47.38	575d ±16.97	665c* ±8.49	6203b ±33.94	5609c* ±60.10	1794d ±30.41	1773c ±12.73	9.03b ±0.14	8.57d* ±0.05	86.8a ±0.07	85.5a ±0.04
Williams	4503d ±67.18	4762b* ±0.71	3979c ±30.41	4123b* ±31.11	525d ±36.77	639c* ±30.41	6369a ±67.88	6265a ±4.95	2391b ±37.48	2142a* ±26.16	8.40d ±0.1	8.87c* ±0.09	87.8a ±0.04	74.5c* ±0.07

^ Year and cultivar interaction was significant ($P<0.05$);[a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$); *Notes the significant difference in mean values between different years ($P<0.05$)

Bannister exhibited the lowest peak viscosity (4369 cP in 2011 and 4385 cP in 2012) and trough viscosity (3477.5 cP in 2011 and 3648 cP in 2012) for both years (Table 4.6). This may be due to a higher lipid and amylose content in this variety in both years. According to Tang and Copeland (2007) starch-lipid complexes can form during the RVA standard testing protocol and this affects the viscosity parameters. Lipids have a tendency to interact with starch granules and prevent their complete hydration, which affects the gelatinisation or pasting properties of starch, resulting in lower peak viscosity (Hüttner et al 2011; Thomas and Atwell 1999, Zhang et al 1997).

The final viscosity (cP) was highest for Mitika (6478 cP) and Williams (6369 cP) for 2011 and Williams (6265 cP) for 2012 (Table 4.6). Williams had the highest β-glucan content, 5.92% in 2011 and 5.93% in 2012, and highest final viscosity value for both years after removing the impact of the covariate, protein. Final viscosity and β-glucan content of oat flour had a positive correlation but the correlation was moderate for the year 2012 (r = 0.4; P>0.05) and strong for the year 2011 (r = 0.807, P>0.05). The weaker correlation between final viscosity and β-glucan content of oat flour (2012) may be because Mitika and Yallara had lower β-glucan content in comparison to Kojonup (2012); however, Mitika and Yallara still had a higher final viscosity than Kojonup in 2012.

Breakdown values ranged from 525 - 892 cP for 2011 and 506 - 894 cP for 2012 (Table 4.6). Choi et al (2012) states that higher β-glucan content in oat flour increases the water binding capacity, which results in formation of more stable hot pastes. High setback is an indicator of retrogradation (Choi et al 2012). Becker et al (2001) reported that wheat and maize products show a positive correlation between coarser particle size and setback. In our research there was a positive relationship between coarser particles and setback (r = 0.858, P < 0.05 for 2011; r = 0.782, P > 0.05 for 2012) (Table 4.6). In 2011 the oat flour pasting temperature did not differ significantly (P>0.05) and ranged from 86.7°C to 87.9°C. In 2012 Kojonup had the highest pasting temperature (87.8°C) and Williams having the lowest (74.5°C) (P<0.05) (Table 4.6).

Overall, Mitika had the highest peak viscosity, trough viscosity and high final viscosity for oat flour although it took more time (Table 4.6, 9.87 min for 2011 and 10.0 min for 2012) to reach the peak viscosity, in comparison to other oat cultivars.

4.5 Conclusion

Our findings show there was significant variability in the quality of the six Western Australian oat cultivars evaluated, attributes which were influenced by genetic and environmental factors. Increased knowledge on the quality criteria is needed for the identification of the Australian oat cultivars which are most suitable for incorporation in noodles in terms of processing ability and noodle quality. This will assist with the utilisation of oats in foods and the marketing of oats to countries such as China. The oat composition, physicochemical properties, and pasting properties were found to differ due to the oat cultivar and growing season. Oat cultivars grown in 2012 had significantly (P<0.05) higher mean values for protein content, lipid content, and had greater volume of finer particle size (<100 μm); and lower values for ash content, starch damage and had lower volume of coarser particle size (>100 μm) in comparison to the same cultivars grown in 2011. Yallara had the highest brightness value and Kojonup had the highest redness values for oat groats, oat flour, and oat flour fractions for both the years. Williams had the lowest brightness value for oat groat, oat flour and oat flour fraction with the exception of 2012 Williams oat flour which was second lowest to Bannister (2011). Furthermore, in comparison to the other oat cultivars, Mitika had highest peak viscosity for 100% oat flour as it had highest amylopectin, protein content and greater volume of finer particle size. Additional research on oat cultivars around the world, such as those from China, is required to understand the potential for high β-glucan oats with superior quality characteristics for incorporation into foods by methods that retain nutritional value is now needed.

In order to set oat cultivar breeding targets for quality traits, it is important to understand how the variation in traits can impact the functionality of oats during processing and how this would affect end product quality. In the next chapter the effect of incorporation of these oat cultivars in white salted noodles will be analysed.

Chapter 5: Study 2- Oat-Wheat Noodle Quality

5.1 Introduction

Oats, *Avena sativa*, are commonly consumed by humans as rolled oats in porridge, rolled and toasted in muesli or incorporated in snack bars (Estévez et al 2005; Aigster et al 2011; Ryan et al 2011). The incorporation of whole grain dehulled oat (groats) flour or flakes into food products to provide health benefits is relatively new and mainly related to high viscous dietary fibre, β-glucan, found in oats (Zhang et al 1998; Flander et al 2007; Aydin and Gocmen 2011; Hüttner et al 2011; Mitra et al 2012) along with other valuable nutrients such as protein, unsaturated fatty acids, minerals, vitamins and phytochemicals (Hampshire 1998; Emmons and Peterson 1999).

The increased consumer awareness of health and health related benefits from foods and food ingredients including oat and barley products containing β-glucan has led to the research and development of new products (Lee and Inglett 2006; Salehifar and Shahedi 2007; Tiwari and Cummins 2009b; Hüttner et al 2011). One of the major challenges of adding oats to different food products however, is the effect the oat addition has on processing and end product quality. Fifty to 150 g of oats is needed to provide an effective amount of β-glucan (3 g) and to make health benefits claim (Wang 2004b, Wood 2007). Oat proteins lack the viscoelastic properties of wheat gluten and this hinders their utilisation to an appreciable extent in traditional wheat based products such as bread, noodles, pasta and biscuits (Zhou et al 2011). Therefore, most commercially available food products have very low content of oats, but some products such as noodles, which have simpler processing steps and formulations have greater potential to include larger amounts of oats in the formulation.

The popularity and simplicity of noodles makes them an ideal base for incorporation of non-traditional ingredients (Twombly et al 2006). Researchers have previously used noodles as a base for the addition of functional ingredients such as garbanzo beans, barley, Nutrim- oat hydrocolloid, seaweed, lupin, sweet potato starch, rye, coconut, barley fiber and oat flour (Kruger et al 1998; Lee et al 1998; Chen et al 2002; Hatcher et al 2005; Inglett et al 2005; Izydorczyk et al 2005; Chang et al 2008; Gunathilake et al 2008; Jayasena et al 2008; Aydin and Gocmen 2011; Mitra et al 2012).

The addition of oats to wheat based noodles improves their nutritional quality due to increased protein and total β-glucan content (Aydin and Gocmen 2011; Mitra et al 2012). However, the main challenge of incorporating oats as an ingredient to make noodles is ensuring the effects on noodle quality are minimised; and this will also depend upon the market requirement for the noodle type being considered. In the case of the popular Japanese white salted noodle known as udon, the requirement is for the noodles to have a clean, bright and creamy appearance and a smooth, soft and elastic texture (Crosbie et al 1990; Konik et al 1992; Hatcher et al 1999). The addition of oats to wheat formulations has been found to negatively affect noodle texture (softer and less elastic) and an increased cooking loss and breakage (Aydin and Gocmen et al 2011; Zhou et al 2011; Mitra et al 2012; Majzoobi et al 2014). These effects on noodle quality are partly due to the dilution or lack of a continuous wheat gluten network (Aydin and Gocmen 2011; Majzoobi et al 2014). Sensory evaluation and colour assessment found oat-wheat noodles were less bright, and more reddish in colour, had a higher speck number and were lower in colour stability (Aydin and Gocmen 2011; Zhou et al 2011; Mitra et al 2012; Majzoobi et al 2014). For Asian noodles, discoloration including darkening and dark spots is unacceptable to consumers (Fuerst et al 2010) and is due to polyphenol oxidase activity, non- enzymatic browning, flour extraction rate, weather damage and genotype (Crosbie and Ross 2004; Fuerst et al 2010). However, some sensory panellists preferred darker coloured oat-wheat noodles with higher specks, as they associated them with increased fibre and healthfulness (Mitra et al 2012).

Despite the reported negative effects of oat flour addition on noodle quality, there remains many opportunities to improve the quality of noodles made with oat flour by ensuring selection of suitable oat cultivars and improving processing techniques (Ames et al 2013). Mitra et al (2012) found that certain oat cultivars were superior in terms of ease of processing and maintaining β-glucan levels during cooking and processing. Seasonal (environmental growing conditions) and varietal (genetic) differences of different oat cultivars affected the composition and physicochemical characteristics of the oat flour such as their pasting properties, β-glucan extractability, molecular weight and fine structure (Shewry et al 2008; Andersson and Börjesdotter 2011; Choi et al 2012; Doehlert and Simsek 2012). Noodles made with 10% oat flour or fine oat bran (extruded) flour, exhibited sensory acceptability closest to that of

wheat flour noodles and that the sensory scores and the quality of noodles decreased as the percentage of oat flour/bran increased (Reungmaneepaitoon et al 2006; Aydin and Gocmen 2011; Mitra et al 2012). However, there are limited studies that have examined both the impact of environment (including year) and genetic variation of oats on the quality of end products (Lapveteläinen et al 2001; Rhymer et al 2005; Hüttner et al 2011; Mitra et al 2012). Rhymer et al (2005) found in a study of five Canadian oat genotypes grown in six different environments that oatmeal texture varied as a result of the starch gel properties. The objective of this research was to investigate the effect of incorporating oat flour from different oat cultivars over two growing seasons on the quality of udon-style white salted noodles (WSN).

5.2 Summary of materials and methods

5.2.1 Materials

Six milling oat cultivars of Western Australia (Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams) that were grown over two different seasons (2011 and 2012) at the same location (Katanning, WA) were selected for this study. Oat cultivars were supplied by the National Oat Breeding Program managed by South Australian Research and Development Institute (SARDI), in collaboration with the Department of Agriculture and Food Western Australia (DAFWA). All tests were performed in at least in duplicates. The wheat flour (control) was standard commercial white salted noodle (udon) flour from Japan.

5.2.2 Noodle processing

Oat grains were processed to oat flour (refer to *section 3.2. Sample preparation* for further details). The official Japanese noodle processing steps used by the Australian Export Grain Innovation Centre (AEGIC) for noodle processing was used to prepare the white salted noodles (WSN) with some modifications for oat-wheat noodle formulation (Mitra et al 2012). (Refer to *section 3.4.2; Table 3.3 Steps involved in WSN udon noodle processing* for further details).

A maximum addition of 30% oat flour to the WSN, without the addition of additives such as gluten or transglutaminase, was previously determined (Mitra et al 2012) so a blend of 30% oat in wheat flour (30% OW) was used in this research. These carefully mixed and homogenised blends were then used for noodle preparation. Noodles were stored in plastic bags at ambient temperature before cooking (within 1 h of processing),

to prepare samples for colour analysis, solid/β-glucan loss in cooking water and β-glucan content of cooked noodles followed by cooking for texture analysis (Refer to *section 3.4.2; Table 3.3 Steps involved in noodle processing* for further details).

5.2.3 β-glucan content of 30% OW

The (1-3) (1-4)-β-D glucan in 30% oat- wheat blend (30% OW) was analysed using a mixed-linkage β-glucan kit assay (Megazyme International Ltd., Wicklow, Ireland) using the approved method 32-23.01 (AACC International 2010) (Refer to *section 3.4.1.30% oat-wheat flour blend and udon flour quality* for further details).

5.2.4 Pasting Properties of 30% OW and control wheat flour

For wheat flour and oat-wheat flour blends (oat: wheat; 30:70) a rapid (13 min) protocol (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used following the AACCI Approved Method 76-21.01 (2010) (Refer to *section 3.4.1. 30% oat-wheat flour blend and udon flour quality* for further details).

5.2.5 Cooking of noodles

Cooking loss, β-glucan content and colour analysis required a 25 g (5 cm) portion of noodles (Approved Method, 66-50.01 AACCI 2010) and for texture analysis a 7 g (7.5 cm) portion of noodles (Sissons 2008) were boiled in a glass beaker containing 250 mL boiling water till its optimum cooking time (OCT) (Solah et al 2007; Sissons et al 2008, Approved Method, 66-50.01 AACCI 2010) (Refer to *section 3.4.3 Cooking of 30% oat-wheat noodles and control wheat noodles* for further details).

5.2.6 β-Glucan analysis of cooked noodles

The (1-3)(1-4)-β-D glucan analysis of cooked noodles was conducted using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland), using the procedure for cooked, toasted or extruded cereal products, based on the principles of approved method 32-23.01 (AACC International 2010 (Refer to section 3.4.4.1 *β-glucan content of cooked noodle* for further details).

5.2.7 Cooking loss in water

The cooking loss of solids from the noodles in cooking water was determined as described in AACCI (2010) Approved Method 66-50.01. The cooking loss of β-glucan in cooking water from the noodles was determined using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland) using the procedure used for the

wort samples (Approved method EBC 8.13.1, 1997). (Refer to section 3.4.4.2 *Solid loss in cooking water and* 3.4.4.3 *β-glucan loss in cooking water* for further details).

5.2.8 Colour analysis of noodle sheet and cooked noodles

Colour analysis of raw noodle sheets and boiled noodles was conducted using a Konica Minolta Chroma Meter (CR-310, Konica Minolta, Tokyo), to measure the CIE *L** (brightness or lightness), *a** (redness and greenness), and *b** (yellowness and blueness) values (Solah et al 2007; Aydin and Gocmen 2011; Mitra et al 2012). CIE *L** of double folded raw noodle sheet was assessed at 0 h and after 24 h on a standard white background (backing tile) (Morris et al 2000; Solah et al 2007) (Refer to section 3.4.4.5 *Colour of noodle sheet and cooked noodles* for further details).

5.2.9 Texture analysis of cooked noodles

The firmness of noodles was analysed using a texture analyser (TA.XT plus) (Stable Micro Systems, UK) equipped with 5 kg load cell and a cutting blade (light knife blade-A/LKB-F). Noodle firmness (height of the peak g/force), or maximum cutting force was determined (Sissons et al 2008) (Refer to section 3.4.4.4 *Firmness of cooked noodles* for further details).

5.3 Statistical analysis

Data was analysed using SPSS Version 21 (SPSS, Chicago, IL). The effects of cultivars, years and the interaction between cultivar by years were assessed using analysis of variance (ANOVA) for 30% OW noodle samples. Comparisons of means for significant effects within the ANOVA were carried out using Least Significant Difference (LSD). Pearson's correlation analysis was applied to find correlations between different variables (oat flour and oat-wheat flour blend composition with noodle and cooking quality). Pearson's correlation coefficients which were strong (r = 0.7 - 0.9) and moderate (r = 0.4 - 0.6) were included in this paper. $P<0.05$ was considered as statistically significant. Error bars represent the standard deviation.

5.4 Results and discussion

5.4.1 Pasting properties of 30% OW and control wheat flour

The pasting properties of wheat flour (control) and 30% oat - wheat blend (30% OW) are shown in Table 5.1 and Figure 5.1. The main effect of year was significant ($P<0.05$) for all RVA parameters for 30% OW with the exception of pasting

temperature and trough. The effect of cultivars was significant (P<0.05) for all RVA parameters of 30% OW. The interaction between year and cultivars was significant (P<0.05) for peak viscosity, trough, breakdown and final viscosity and not significant (P>0.05) for setback, peak time and pasting temperature (refer to *Appendix 6; Table 6A* for further details).

The peak viscosity, trough, final viscosity and setback values were significantly higher (P<0.05) for 30% OW in comparison to the wheat flour for all oat cultivars (Table 5.1). Incorporation of 30% oat flour in wheat flour increased the lipid content, particle size and β-glucan content (refer to *Table 4.1* in *Chapter 4)* and these components were significantly higher in oat flour than in the wheat flour. The high peak viscosity may be a result of the amylose - lipid complex which delays the swelling of the starch granules; as the complex dissociates when high temperatures are reached and this delayed swelling of oat starch results in a paste with high viscosity and high stability at high temperatures (Lim et al 1992; Zhou et al 1998b).

The coarser particle size and β-glucan content of oat flour (refer to *Table 4.1* in *Chapter 4*) incorporated in wheat flour could have contributed to the increase in viscosity of the blends. Coarser particles cause higher final viscosity (Izdorczyk et al 2005; Stevenson et al 2007). The β-glucan content in 30% OW may have increased the water absorption resulting in higher peak viscosity, trough and final viscosity in comparison to the wheat control (Choi et al 2012). A moderate positive correlation was noted between final viscosity and β-glucan content of 30% OW (r = 0.686, P>0.05 for 2011; r = 0.651, P>0.05 for 2012).

Table 5.1 RVA pasting properties of 30% oat-wheat blends and control wheat flour

Cultivars	Peak^ (cP)		Trough^ (cP)		Breakdown^ (cP)		Final Viscosity^ (cP)		Setback # (cP)		Peak Time # (Min)		Pasting Temperature # (°C)	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	3702a ±4.95	3667a* ±10.96	2137a ±3.50	2151a ±27.93	1574b ±0.71	1516b* ±38.90	3596a ±10.25	3550a* ±7.42	1459 ±6.72	1399 ±20.51	6.12 ±0.21	6.20 ±0.05	69.5 ±0.05	69.9 ±0.00
Kojonup	3592c ±17.32	3577b ±12.02	1987d ±1.06	2052b* ±38.54	1606a ±16.26	1525b* ±26.52	3414d ±13.79	3461c* ±30.76	1427 ±12.73	1410 ±7.78	6.00 ±0.00	6.10 ±0.04	70.3 ±0.02	69.7 ±0.32
Yallara	3567d ±5.67	3575b ±6.72	2008d ±7.07	1999.25c ±2.47	1559b ±12.73	1576a ±4.24	3425cd ±12.37	3385d* ±12.02	1417 ±5.30	1385 ±14.50	6.02 ±0.03	6.05 ±0.03	69.7 ±0.32	69.5 ±0.00
Bannister	3532e ±15.56	3511d* ±1.40	1951e ±6.01	1989c* ±11.67	1581ab ±9.55	1521b* ±13.08	3341e ±7.78	3355e ±12.37	1389 ±1.77	1366 ±0.71	5.98 ±0.03	6.05 ±0.03	69.5 ±0.04	69.6 ±0.00
Carrolup	3619b ±0.71	3500d* ±0.35	2038c ±10.25	1998c* ±9.19	1581ab ±9.55	1503bc* ±8.83	3451c ±8.49	3388d* ±32.17	1412 ±1.77	1390 ±22.98	6.05 ±0.02	6.04 ±0.00	69.5 ±0.02	69.3 ±0.25
Williams	3580cd ±6.70	3545c* ±6.01	2080b ±2.12	2061b ±0.71	1501d ±4.60	1484c ±6.72	3533b ±5.30	3500b* ±1.77	1454 ±7.42	1439 ±1.06	5.97 ±0.00	6.00 ±0.00	69.5 ±0.04	69.1 ±0.57
Control	3257f ±7.10	3257e ±7.10	1727f ±6.36	1727d ±6.36	1531c ±0.71	1531b ±0.71	3035f ±3.54	3035f ±3.54	1308 ±9.90	1308 ±9.90	6.00 ±0.00	6.00 ±0.00	68.7 ±0.00	68.7 ±0.00

^ Year and cultivar interaction was significant ($P<0.05$); # Year and cultivar interaction was not significant ($P>0.05$);[a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$)

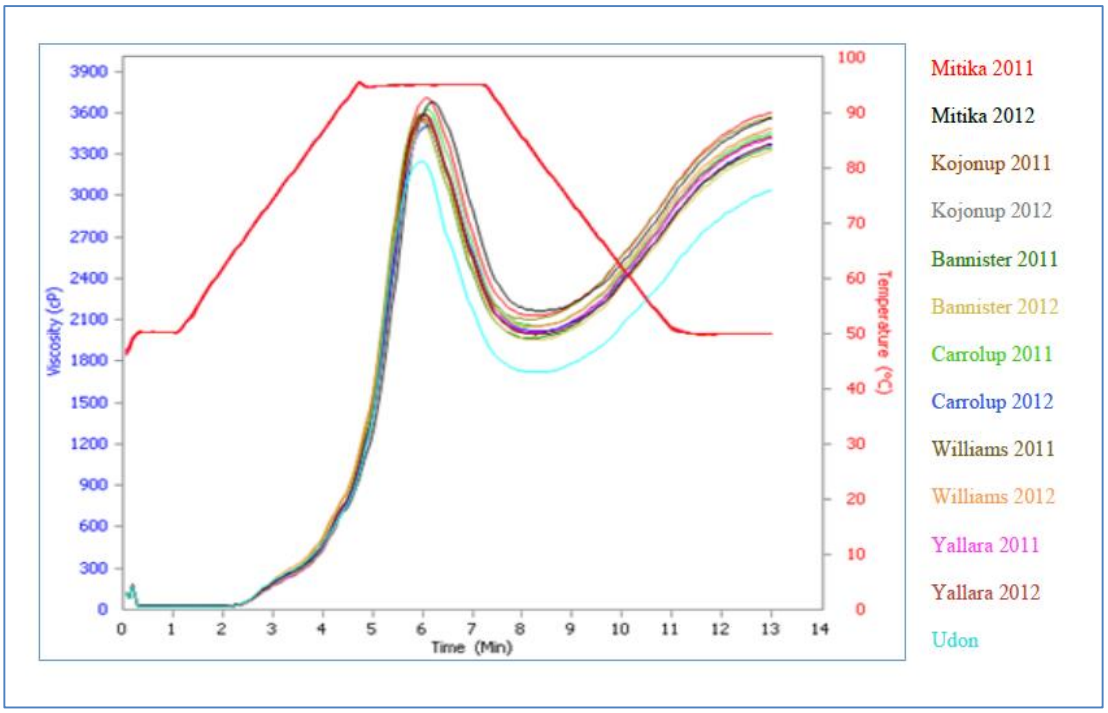

Figure 5.1 RVA Pasting Properties of 30% OW and control (udon) wheat flour

Overall, Mitika had the highest peak viscosity, trough viscosity and high final viscosity for 30% OW, although it took more time (Refer to *Appendix 6; Table 6A* for further details, 6.16 min for 30% OW samples - average of same cultivar over 2 years) to reach the peak viscosity, in comparison to other oat cultivar.

The 30% OW samples were processed into noodles and their quality was assessed. High quality Asian noodles must have an acceptable colour, brightness and mouthfeel of the boiled noodles (Hatcher et al 2002).

5.4.2 Noodle sheet colour (at 0 h and 24 h)

Noodle sheet colour for 30% OW samples were noted at 0 h and after keeping the raw sheets at 25°C for 24 h was noted and presented in Tables 5.2 and 5.3.

Table 5.2 CIE (*L*a*b) colour values of 30% OW noodle sheet (0 h)**

Cultivars	CIE L* value of noodle sheet^		CIE a* value of noodle sheet^		CIE b* value of noodles sheet#	
	2011	2012	2011	2012	2011	2012
Mitika	78.73a ± 0.11	78.94b ± 0.02	1.95c ±0.004	1.83b ± 0.057	15.87 ± 0.067	17.12 ± 0.14
Kojonup	77.11b ± 0.29	78.17c * ± 0.48	2.51a ± 0.090	2.35a * ± 0.074	14.98 ± 0.45	16.53 ± 0.40
Yallara	78.76a ± 0.24	79.72a * ± 0.1	1.81c ± 0.044	1.31d * ± 0.014	14.97 ± 0.15	16.4 ± 0.31
Bannister	76.11c ± 0.71	78.20c * ± 0.17	2.26b ± 0.042	1.96b * ± 0.087	15.75 ± 0.055	17.95 ± 0.002
Carrolup	77.46b ± 0.13	78.39bc* ± 0.38	2.22b ± 0.028	1.94b * ± 0.13	15.58 ± 0.62	16.79 ± 0.23
Williams	77.29b ± 0.37	78.01c ± 0.27	1.89c ± 0.044	1.66c * ± 0.05	14.91 ± 0.06	16 .00 ±0.17

^ Year and cultivar interaction was significant (P<0.05); [a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05);*Notes the significant difference in mean values between different years (P<0.05); # Year and cultivar interaction was not significant (P>0.05)

Addition of oat flour to wheat flour at a proportion of 30% produced noodle sheets (0 h) which were significantly lower in CIE *L** (brightness) and CIE *b**(yellowness) values and higher in CIE *a** (redness) values in comparison to control wheat flour (L* 85.11; a* - 0.29; b*20.03). Similar results were noted in previous research (Mitra et al 2012) (Refer to *Appendix 9* for photographs). CIE (*L*a*b**) colour values (Table 5.2) of 30% OW noodle sheet (0 h) show that Yallara (*L**: 78.76 for 2011; 79.72 for 2012) had the highest brightness (CIE *L**) values followed by Mitika (*L**: 78.73 for 2011; 78.94 for 2012) in comparison to other oat cultivars. CIE *a** value (redness) was highest for Kojonup (*a**: 2.51 for 2011; 2.35 for 2012) and lowest for Yallara (*a**:1.81 for 2011; 1.31 for 2012) for both years. Mitika (CIE *b** 16.49) and Bannister (CIE *b** 16.85) had the highest CIE *b** (yellowness) value (average across two years) and Williams (CIE *b** 15.45) had the lowest CIE *b** value (average across two years).

Table 5.3 CIE (L*a*b*) colour values of 30% OW noodle sheet (24 h)

Cultivars	CIE L* value of noodle sheet^		CIE a* value of noodle sheet^		CIE b* value of noodle sheet^	
	2011	2012	2011	2012	2011	2012
Mitika	67.96a ± 0.27	68.77b* ±0.64	2.89d ± 0.00	2.71c* ± 0.18	19.89a ± 0.09	21.16b* ± 0.16
Kojonup	65.75b ±0.34	67.57c* ± 0.05	3.72a ± 0.03	3.46a* ± 0.07	19.18b ± 0.17	20.53cd* ± 0.39
Yallara	67.67a ± 0.11	69.76a* ± 0.51	2.75d ± 0.02	2.21d* ± 0.14	19.00bc ± 0.06	20.41d* ± 0.033
Bannister	64.15c ± 0.6	67.19c* ± 0.39	3.49b ± 0.13	3.37a ± 0.11	19.20bc ± 0.07	21.56a* ± 0.091
Carrolup	65.88b ± 0.24	67.02cd* ± 0.52	3.31c ± 0.14	3.12b* ± 0.02	19.95a ± 0.17	20.88bc* ±0.16
Williams	65.40b ± 0.24	66.42d* ± 0.14	3.35bc ± 0.12	3.06b* ± 0.07	18.63c ± 0.15	19.69e* ±0.16

^ Year and cultivar interaction was significant (P<0.05); [a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05);*Notes the significant difference in mean values between different years (P<0.05)

Table 5.3 shows the CIE (*L*a*b**) colour values for noodle sheet after storing it for 24 h. The control wheat flour noodle sheet CIE *L*a** and *b** values (24 h) (*L** 79.4; *a**0.18; *b**23.73) were significantly different to 30% OW noodle sheet (24 h), however, both showed a reduction in CIE *L** values and increases in CIE *a* * and CIE *b** values. Visually, over 24 h the noodle sheets became darker with more specks. These darkening of noodle sheets over time are mainly due to enzymatic and non-enzymatic browning reactions (Fuerst et al 2010) and are discussed in the following section on colour stability. For CIE *L** (brightness) colour values of 30% OW noodle sheet (24 h), Yallara (*L**:67.67 for 2011; 69.76 for 2012) and Mitika (*L**: 67.96 for 2011 and 68.77 for 2012) had higher values in comparison to other oat cultivars. The CIE *a* *(redness) value for 30% OW noodle sheet (24 h) was highest for Kojonup (3.72 for 2011 and 3.46 for 2012) and lowest for Yallara (2.75 for 2011 and 2.21 for 2012). The CIE *b** (yellowness) value was highest for Mitika (19.89), Carrolup (19.95) for 2011 and Bannister (21.56) for 2012, and lowest for Williams (18.63 for 2011; 19.69 for 2012) in comparison to other oat cultivars. The main effect of year of cultivation, cultivar and interaction between the year of cultivation and cultivar for CIE (*L*a*b**)

colour values of 30% OW noodle sheet at 0 h and 24 h was significant (P<0.05), except the effect of interaction between the year of cultivation and cultivar was not significant (P >0.05) for CIE b* value of 30% OW noodle sheet at 0 h.

5.4.3 Colour stability of noodle sheet

Darkening of raw noodles over time is unacceptable by consumers (Fuerst et al 2010). Although darkening of noodles can be arrested by boiling of the raw noodles still minimal darkening of raw noodles over 48 h is desirable (Ram and Mishra 2010). Therefore the colour stability in terms of brightness (CIE L*) value of raw noodle is important.

The change in the CIE L* values over 24 h was minimal (high colour stability) for the control WSN raw noodle sheet (ΔL* 5.74) in comparison to 30% OW raw noodle sheet (Figure 5.2). The incorporation of oat flour reduced noodle colour stability significantly (P<0.05). The main effect of cultivar and year of cultivation on CIE ΔL* (0-24 h) was significant (P<0.05), but the interaction of cultivar and year of cultivation was not significant (P>0.05) for colour stability.

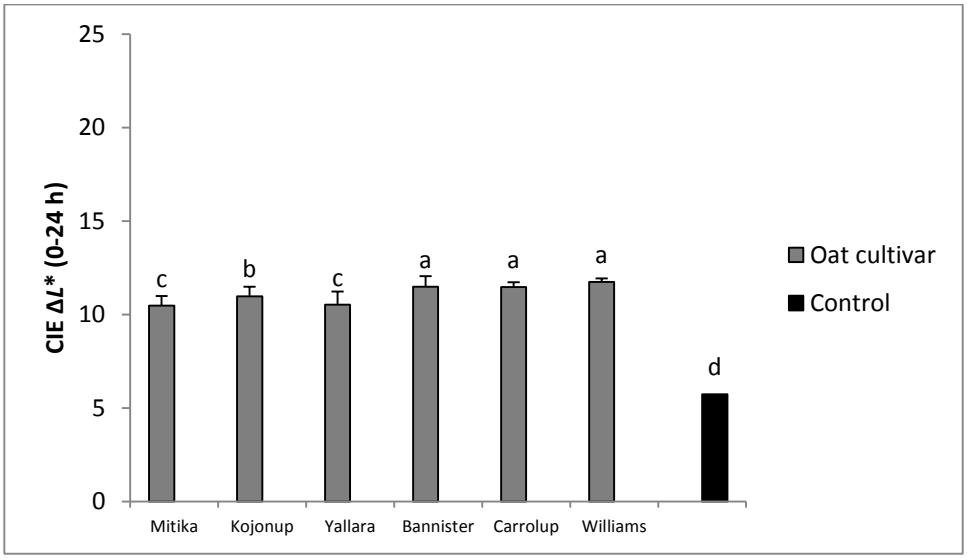

Figure 5.2 CIE (Δ L*) value of noodle sheet colour change (0-24 h)

(Average of a cultivar over 2 years); bars labelled with different letters are significantly different (P<0.05); error bars indicate standard deviation

For colour stability of 30% OW raw noodle sheet, the average across all cultivars grown in 2012 was significantly higher than the average of all cultivars grown in 2011.

Mitika (ΔL^* 10.47) and Yallara (ΔL^* 10.53) (average across two years) (Refer to *Appendix 6; Table 6B* for further details) had the least colour change (ΔL^*) over a period of 24 h; thus increased colour stability in comparison to other oat cultivars (Figure 5.2).

The darkening of noodles over time can be associated with bran specks and enzymatic browning by polyphenol oxidase (PPO) (Miskelly 1984; Baik et al 1995 Fuerst et al 2010). These enzymes oxidise phenols to quinones which are then converted to dark coloured pigments due to self-polymerisation or by interactions with other proteins (Miskelly 1984; Ram and Mishra 2010). The wheat flour added would have the PPO enzyme, which could have contributed partly to the enzymatic browning over 24 h. However, the oat flour samples were heat treated which could have deactivated the PPO, but a variation in darkening amongst oat cultivars was observed. This indicates that other than the PPO from wheat flour other factors were responsible for the darkening in 24 h.

The difference in colour stability in terms of brightness between oat cultivars could have been due to the variation in the survival of some residual PPO in oats after heat treatment or due to non-polyphenol oxidase reactions which mostly are non- enzymatic reactions in the noodle sheets. Previous studies have shown incomplete prevention of darkening even after the use of PPO inhibitors in wheat noodles and this suggests that other than PPO there are other non- PPO darkening mechanisms (Asenstorfer et al 2009; Fuerst et al 2010). However, the exact mechanism for non- enzymatic darkening in wheat noodles is unknown and some suggested reasons given are: increased levels of the darkening substrates, autooxidation of phenolic compounds, reactions of aromatic amino acids of proteins, metallic cations acting as catalysts and low temperature Maillard (amine-carbonyl) reaction which produces coloured chromophore (Asenstorfer et al 2009; Fuerst et al 2010).

5.4.4 Noodle colour

Table 5.4 CIE (*L*a*b) of boiled noodles incorporated with 30% oat flour**

Cultivars	CIE *L** value of boiled noodles^		CIE *a** value of boiled noodles^		CIE *b** value of boiled noodles^	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	68.62b ±0.16	70.37b* ±0.17	1.56d ±0.09	1.34c* ±0.02	12.96b ±0.16	13.66b* ±0.16
Kojonup	66.66c ±0.23	68.67d* ±0.21	2.29a ±0.11	1.76a* ±0.01	12.22cd ±0.02	13.21c* ±0.19
Yallara	66.97c ±0.06	69.29c* ±0.36	1.66cd ±0.01	0.87e* ±0.07	12.42c ±0.15	13.4bc* ±0.25
Bannister	65.46d ±0.05	68.96cd* ±0.06	1.77bc ±0.15	1.29cd* ±0.07	11.88d ±0.35	13.51bc* ±0.04
Carrolup	66.67c ±0.04	68.57d* ±0.28	1.91b ±0.02	1.6b* ±0.02	12.98b ±0.32	13.72b* ±0.27
Williams	65.83d ±0.26	68.18e* ±0.33	1.66cd ±0.01	1.16d* ±0.01	11.97d ±0.23	12.27d ±0.05
Control	76.94a ±0.01	76.94a ±0.01	-1.13e ±0.04	-1.13f ±0.04	16.17a ±0.12	16.17a ±0.12

^ Year and cultivar interaction was significant ($P<0.05$); [a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$)

CIE (*L*a*b**) colour values of boiled noodles after 15 min are reported in Table 5.4 (Refer to *Appendix 10* for photographs). The addition oat flour affected noodle colour and it was significantly different ($P<0.05$) from the control WSN for all blends. The addition of 30% oat flour in WSN reduced the CIE *L** (brightness) values and *b** (yellowness) values and increased the *a** (redness) values in comparison to the WSN, as found in earlier research (Mitra et al 2012; Majzoobi et al 2014).

The main effect of year of cultivation (environment), cultivar (genotype), and interaction between the year of cultivation and cultivar were significant ($P<0.05$) for CIE (*L*a*b**) values of boiled 30% oat-wheat noodles (30% OW noodles). The CIE colour values for 30% OW noodles made with the 2012 grown oat cultivars were significantly different ($P<0.05$) to the CIE colour values of 30% OW noodles with the same oat cultivar grown in 2011. For the 2012 growing year, each oat cultivar produced 30% OW noodles with higher values for CIE *L** and CIE *b** and lower values for CIE *a** in comparison to the same oat cultivar grown in 2011.

The total amount of rainfall for the year 2011 was very high (668.4 mm) in comparison to 2012 (481.0 mm) in Western Australia (Katanning) (refer to *Appendices 1* for furthur details). High rainfall during October and November (refer to *Appendices 1* for furthur details), just before harvest could have contributed to the discolouration of the 2011 grains and contributed to the differences in colour (Bureau of Meteorology 2013). High humidity or temperature during harvest or grain filling can be associated with discolouration of grains (stained grains) which can be caused by mould growth or activation of peroxidase enzyme but the exact cause is not known (Marano et al 2012; AWB 2014).

Mitika (30% OW) noodles were the whitest/brightest (CIE *L** 68.62 and 70.37 for 2011 and 2012 respectively) with fewer specks than the other cultivars. Mitika and Carrolup were the most yellow (CIE *b** 12.96 and 12.98 respectively for 2011, and 13.66 and 13.72 respectively for 2012) noodles. Kojonup was highest in redness (CIE *a** 2.29 for 2011 and 1.76 for 2012) for both years (Table 5.4). Yallara had the highest brightness value for oat grains, oat flour, oat flour fraction (refer to *Tables 4.2 - 4.5* in *Chapter 4)* and 30% OW noodle sheet (at 0 h). Whole grain flour colour is influenced not only by the colour of the endosperm, but also the colour of the bran, which would interact with other components/factors of the flour (e.g. residual enzymes, non-enzymatic reactions, flour particle size, protein, lutein and lutein esters) during noodle processing and boiling and affect the end product colour (Miskelly 1984; Hou and Kruk 1998; Hatcher et al 2002; Salehifar and Shahedi 2007; Mares et al 2008; Asenstorfer et al 2009; Fuerst et al 2010). A strong positive correlation (r = 0.956, P<0.05 for 2011 and r = 0.718, P>0.05 for 2012) was observed between CIE *L** value of oat groat (*refer to Table 4.2 in chapter 4*) and *L** value of oat flour (refer *to Table 4.3* in *Chapter 4*). CIE *L** groat colour value of Mitika (CIE *L** 55.85 for 2011; CIE *L** 58.17 for 2012) was second highest after Yallara (CIE *L** 56.47 for 2011; CIE *L** 61.93 for 2012) and lowest for Williams (CIE *L**52.98 for 2011; CIE *L** 56.70 for 2012) for both years.

5.4.5 Texture (firmness) of cooked noodles

The firmness of WSN (1205 g/force) was significantly higher (P<0.05) than the 30% OW noodles whose firmness values ranged from 852 - 896 g/force for 2011 and 943 - 1012 g/force for 2012 (refer to *Appendix 6; Table 6C* for further details). Other researchers have noted that addition of non- wheat flour (bean flour, oat, lupin) and

seaweeds mixed with wheat flour interfered in the formation of gluten and hence, it weakened the texture of noodles (Wang 2004b, Lagasse et al 2006; Chang and Wu 2008). In our research study, the oat flour containing β-glucan is added with wheat flour to make noodles. The β-glucan in oat flour is viscous and it may hold more water and cause softening of noodles or pasta as reported by Brennan and Tudorica (2007). Although the time to peak viscosity (RVA) was significantly different between different oat cultivars, the cooking time for the 30% OW noodles samples did not differ between cultivars and years. The optimum cooking time (OCT) for the OW noodle samples was approximately 7 min and for the WSN OCT was approximately 8 min. This difference in cooking time is likely due to the addition of oat flour which may have resulted in an increase in water absorption which resulted in faster penetration of heat and moisture (Izydorczyk et al 2005).

The interaction between genotype and environment (year of cultivation) and cultivar main effect were not significant ($P>0.05$) but the main effect of year was significant ($P<0.05$), in firmness of 30% OW noodles. All cultivars grown in 2012 (average across all cultivar, 988.54 g/force) were significantly firmer ($P<0.05$) than in 2011 (average across all cultivar, 876.42 g/force) (Mean difference 112.1 g/ force; 95% class interval for difference: lower limit 87.42 g/force and upper limit 136.83 g/force). In our research the samples grown in 2012 had a high percentage of finer particles but less starch damage compared to samples grown in 2011, so it is likely that the lower starch damage and therefore lower water uptake influenced firmness. Higher pasting peak viscosity of flour relates to the desired softer texture needed for Japanese WSN (Crosbie 1991; Black et al 2000), but in this research there was no difference in the textural properties of oat cultivars for both years.

5.4.6 Solid loss in cooking water

Noodle samples with oat flour had a significantly higher cooking loss percentage ($P<0.05$) in comparison to the WSN (3.80%) as shown in Figure 5.3

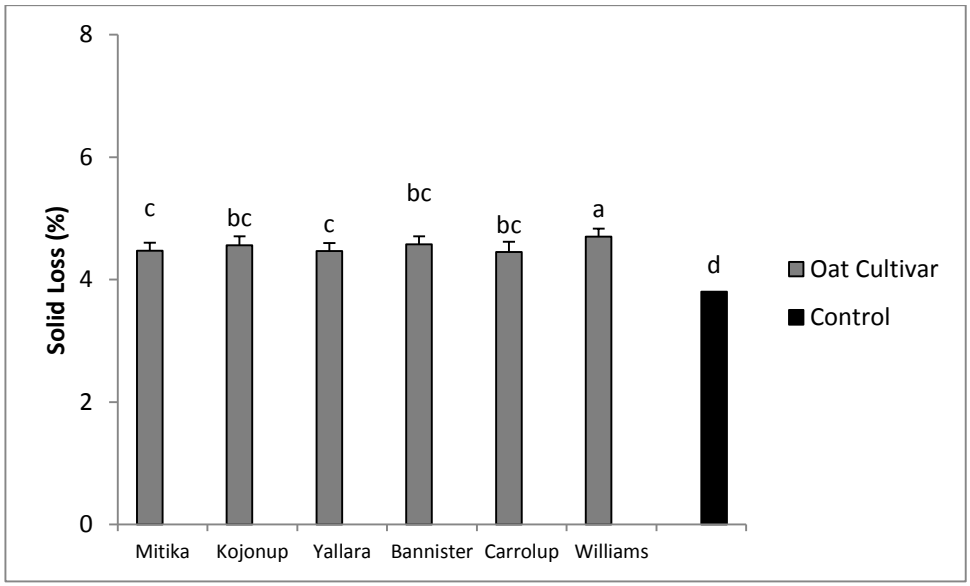

Figure 5.3 Cooking loss (%) (solid loss in cooking water) of 30% OW noodles

(Average of a cultivar over two years); bars labelled with different letters are significantly different (P<0.05); error bars indicate standard deviation

Cooking loss of solids can be caused by the disruption or weakening of the starch - protein matrix (Tudorica et al 2002; Izydorczyk et al 2005). Dilution of the gluten content and change in the gluten development due to oat flour incorporation could be the cause for this (Sabanis et al 2006; Aydin and Gocmen 2011; Majzoobi et al 2014). The other reason for higher cooking loss in oat-wheat noodles could be related to the higher levels of water soluble components in oat flour, particularly β-glucan and protein components that might have leeched out and entered the cooking water (Majzoobi et al 2014).

The main effect of cultivar (average over two years) and year (average across all cultivars grown in a year) was significant ($P<0.05$), but the interaction between year and cultivar was not significant ($P>0.05$) for solid loss in cooking water (refer to *Appendix 6; Table 6D* for further details). The average values over two years (Figure 5.3) for oat cultivars show that the solid loss was lowest for Mitika (4.47%), and Yallara (4.47%) and was significantly lower ($P<0.05$) than Williams (4.70%). A strong positive correlation was noted between coarser particle size (>100µm) and solid loss for both the years (r = 0.842, $P<0.01$ for 2011 and r = 0.961, $P< 0.01$ for 2012). Williams had the highest percentage of coarser particle size (>100µm) (refer to *Table 4.1* in *Chapter 4)* and highest solids loss for both the years in comparison to other oat cultivars. In contrast, Hatcher et al (2008) found cooking loss was not affected by

either starch damage or particle size regardless of alkaline reagent, indicating that other factors are responsible.

5.4.7 β-Glucan loss in cooking water

The main effect of cultivar (average over two years) and interaction between cultivar and environment (year of cultivation) were significant (P<0.05) for β-glucan content of 30% OW, 30% OW noodle, cooking water and percent decrease of β-glucan from 30% OW after processing into 30% OW noodles. The main effect of year (average across all cultivars grown in a year) was also significant for all these parameters except for the β-glucan content of 30% OW noodles.

Table 5.5 β-glucan content of 30% OW, 30% OW noodle, cooking water and percent decrease in β-glucan of 30% OW after processing

Cultivars	β-glucan % of 30% OW^ (dwb) Pre processing		β-glucan% of 30% OW^ (dwb) Noodle after Processing		% decrease in β-glucan of 30% OW after processing^		β-glucan content in cooking water mg/L^	
	2011	**2012**	**2011**	**2012**	**2011**	**2012**	**2011**	**2012**
Mitika	1.73b ±0.02	1.65c* ±0.05	1.52b ±0.04	1.43c* ±0.01	12.2cd ±0.97	13.3a ±3.04	18.2b ±0.04	17.6b* ±0.07
Kojonup	1.70b ±0.01	1.70b ±0.00	1.42c ±0.04	1.50b* ±0.00	16.5b ±1.70	11.8a* ±0.00	16.3d ±0.14	16.1c ±0.18
Yallara	1.52d ±0.01	1.52e ±0.02	1.36d ±0.02	1.36d ±0.06	10.9d ±0.67	10.3a ±2.47	16.2d ±0.24	16.1c ±0.14
Bannister	1.56c ±0.01	1.57d ±0.00	1.24e ±0.01	1.37d* ±0.05	20.6a ±0.09	13.1a* ±3.15	17.1c ±0.64	16.4c* ±0.28
Carrolup	1.71b ±0.00	1.59d* ±0.01	1.45c ±0.01	1.38cd* ±0.07	15.5bc ±0.41	12.9a ±4.07	17.3c ±0.40	17.2b ±0.11
Williams	1.87a ±0.02	1.87a ±0.00	1.66a ±0.03	1.65a ±0.03	11.0d ±0.50	11.8a ±1.5	20.7a ±0.20	19.8a* ±0.40

^Year and cultivar interaction was significant (P<0.05); [a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); *Notes the significant difference in mean values between different years (P<0.05)

The β-glucan content 30% OW (Table 5.5) was highest for the Williams and lowest for Yallara for both years as previously noted for the β-glucan content of 100% oat flour (refer to *Table 4.1* in *Chapter 4*). The loss of β-glucan after cooking of noodles enriched with fibre rich fraction of barley has been previously reported of up to 2.6% by Izydorczyk et al (2005) and up to 35% by Mitra et al (2012) who found noodles enriched with 30% oat flour lost 0.83 g /100g of the original 2.35 g/100g β-glucan during cooking. The particle size of the oat flour in Mitra et al (2012) study was coarser

(used sieve size of 500 µm for milling) in comparison to the particle size of the oat flour (size of 250 µm for milling) used for this study.

One or more of the many processing steps may be responsible for the loss of β-glucan. Majzoobi et al (2014) research on noodle surface incorporated with oat flour showed that there was a negative effect on surface uniformity such as cracks and pitting on the surface, resulting in loss of water soluble component into the cooking water.

The percentage decrease of β-glucan was highest for Bannister (20.6%) for 2011 and Mitika (13.3%) for 2012, and lowest for Yallara (10.9% for 2011 and 10.3% for 2012) for both years (Table 5.5). The differences in the percent loss of β-glucan due to processing between oat cultivars and seasonal differences, found in our research, may be because of the different molecular weights of β-glucan, solubility of β-glucan, and composition in oats which varied between cultivars and the environment in which they were grown (Johansson et al 2000; Wood 2007; Yao et al 2007).

There were very small amounts of β-glucan in noodle cooking water (Table 5.5) due to leaching from the noodle surface and the solid loss. The β-glucan content of cooking water ranged from 16.2 mg/L for Yallara to 20.7 mg/L for Williams in 2011, 16.1 mg/L for Yallara, and 19.8 mg/L for Williams in 2012 (Table 5.5). A strong positive correlation was found between β-glucan content of 30% OW and β-glucan content of noodle cooking water (r = 0.82, P < 0.05 for 2011; r = 0.819, P<0.05 for 2012). A positive correlation was noted between β-glucan content of cooking water with percentage of coarser (refer to *Table 4.1* in *Chapter 4)* particle size (r = 0.785, P>0.05 for 2011; r = 0.54, P>0.05 for 2012); and with solid loss (r = 0.692, P>0.05 for 2011; r = 0.445, P>0.05 for 2012).

5.5 Conclusion

In this study it was noted that variability in quality of six Western Australian oat cultivars due to genetic and environmental factors had a significant impact on 30% OW noodle quality. However, noodle quality of the oat-wheat noodles identified season (environment) as having stronger impact on textural firmness than the genetic impact of cultivars. Mitika flour blends were easier to handle while processing into noodles. Oat cultivars Mitika and Yallara incorporated 30% OW, produced the brightest noodles with fewer specks and had the greatest colour stability. Although

Williams oat flour and 30% OW had the highest β-glucan content, it also had the highest percentage of coarser particle size and produced noodles with reduced brightness, increased specks and highest β-glucan loss and solid loss in cooking water. This research showed the characteristics of Mitika made it suitable for incorporation in WSN noodles when compared to the other oat cultivars grown in the same year in terms of β-glucan content and stability, pasting properties, noodle colour, colour stability and ease of processing.

This study gives us valuable information regarding 30% OW noodle quality. However, it is important to understand how incorporation of oat flour in wheat flour and its processing into 30% OW noodles would have an effect on oat β-glucan structure and functional properties. Since, viscosity developed by β-glucan is an important aspect related to health, the impact of processing on β-glucan viscosity and related parameters such as molecular weight and solubility should be investigated. This is covered in the following chapter.

Chapter 6: Study 3- Effects of Processing on Oat β - Glucan and its Physicochemical Properties

6.1 Introduction

Oats have been identified as a food with health benefits mainly due to the viscous soluble fibre (1,3)(1,4)-β-glucan found in the endosperm cell wall (Johansson et al 2000; Daou and Zhang 2012; Gamel et al 2012; Xu 2012). Consumption of oat dietary fibre can effectively lower serum cholesterol, moderate postprandial blood glucose levels and insulin levels and help control weight through improved satiety (Ripsin et al 1992; Hallfrisch et al 1995; Davy et al 2002; Biorklund et al 2005; Wood 2007; Regand et al 2011; Daou and Zhang 2012; Stewart and McDougall 2014; Wang and Ellis 2014; Whitehead et al 2014). The European Union health claims for barley and oat β-glucans require the consumption of 4 g β-glucans/ 30 g of available carbohydrate for the reduction of post-prandial glycaemia (EFSA 2011; Harland 2014); whereas the U.S. Food and Drug Administration (FDA) (2014) specifies an intake of 3 g or more β-glucan per day for reduction of blood cholesterol. These recommended quantities of β-glucan cannot solely ensure the physiological efficiency. The amount and molecular weight of β-glucan solubilised in the gastro intestinal tract also has a significant contribution to the blood cholesterol and blood glucose reducing properties (Wang and Ellis 2014).

The mechanism/s by which β-glucan achieves these health benefits is not fully understood but its effect on the viscosity of the upper gastrointestinal tract is thought to be important (Rimsten et al 2003; Ames et al 2015). Hence it is important to understand which factors influence the viscosity of β-glucan. The concentration, solubilisation and molecular weight of β-glucan are all important factors (Beer et al 1997a; Rimsten et al 2003; Ajithkumar et al 2005; Wolever et al 2010). External influences, such as processing of food products prior to digestion, might have an impact on the factors that affect β-glucan viscosity, and therefore may have the potential to influence the physiological effects derived from β-glucan (Tosh et al 2008; Tosh et al 2010). Although many in vivo studies (Granfeldt et al 2008; Hblebowicz et al 2008; Charlton et al 2012; Zhang et al 2012; McGeoch et al 2013;) have shown a positive influence of β-glucan from oat incorporated products on health, the magnitude

of this effect is variable and sometimes not significant (Lovegrove et al 2000; Chen et al 2006).

This variability in physiological activity of β-glucan from oat products can be explained by other factors apart from the β-glucan content and they are MW and extractability/solubility of β-glucan (Regand et al 2009; Wang and Ellis 2014). The physical state of β-glucan, in raw material, β-glucanase activity, processing and storage conditions affect the MW and solubility of β-glucan (Beer et al 1997b; Åman et al 2004; Andersson et al 2004 ; Andersson et al 2008; Regand et al 2009; Tosh et al 2010). These conditions may change the physicochemical properties of β-glucan in food such as poor solubility or reduced molecular weight of the food consumed and reduced ability of β-glucan to form a viscous solution in the gut (Beer et al 1997b; Tosh et al 2010; Wang and Ellis 2014).

Heat treated groats and flakes, flour and bran prepared from it has no β-glucanase activity, thus they have intact high MW β-glucan with high viscosity in comparison to the untreated oats (Antilla et al 2004; Åman et al 2004; Ames et al 2015). Also, dry processing of groats like, milling, sieving and rolling does not impact the β-glucan significantly (Åman et al 2004). However, baking and freezing treatments show a reduction in solubility and MW of β-glucan (Beer et al 1997b). Inclusion of oat flour in wheat flour for preparation of pasta or bread also causes depolymerisation and reduction in solubility of β-glucan due to the impact of β-glucanase present in the wheat flour (Regand et al 2009; Gamel et al 2015). Extrusion process involves application of high heat, pressure and sheer force which increased the solubility of β-glucan in extruded products. However with more severe conditions for extrusion the β-glucan MW decreased by > 10 folds (Tosh et al 2010).

Recently in human feeding trials the association of β-glucan properties and its physiological effects on human clinical trials have been explored (Ames et al 2015). Results from these trials revealed that food containing high MW and/or high solubility of β-glucan more effectively lowered cholesterol and glycemic response in human subjects (Wolever et al 2010; Tosh 2013). It is therefore important to study the impact of processing on β-glucan physicochemical properties, particularly concentration, solubility, molecular weight and their impact on viscosity.

In vitro methods have been designed to determine the physiological effect and behaviour of β-glucan by mimicking the oral, gastric and upper intestinal digestive processes in different ways (Beer et al 1997b; Cleary et al 2007; Tosh et al 2010; Wang and Ellis 2014). In literature the term solubility is interchangeably with extractability (Wang and Ellis 2014). Several techniques have been developed to study the solubility/extractability and molecular weight of β-glucan using physiological extraction and hot water extraction (Anderson et al 1978; Wood et al 1991a; Beer et al 1996; Beer et al 1997b). Mild extraction techniques solubilise 30-70% of the β-glucan and the use of a more vigorous reagent (NaOH) and conditions extract total β-glucan (Wood et al 1991a; Bhatty 1993; Beer et al 1997b; Ajithkumar et al 2005). When physiological extraction is conducted at 37°C with gut enzymes, the viscosity of the β-glucan is reported to correspond to the bioactivity of the β-glucan (Tosh et al 2010).

Beer et al (1997b) established an enzymatic extraction method for barley and oat β-glucan, in which digestive enzymes including human salivary α-amylase, porcine pepsin and pancreatin was used at human body temperature (37°C) to obtain a physiological extract with soluble β-glucan. This method was designed to simulate the physiological conditions of digestion in the upper gastro-intestinal tract (Tosh et al 2008; Wolever et al 2010) but it was time consuming and complex (Gamel et al 2012). Gamel et al (2012) developed a method using the Rapid Visco Analyser (RVA) (to measure viscosity of β-glucan under controlled temperature conditions (body temperature at 37°C), shear rate of 160 rpm (equivalent to the shear rate of 54 sec^{-1}, which is similar to the shear rate occurring in the gut (Booth and Bason 2007) with a combination of digestive enzymes. This method developed by Gamel et al (2012) correlated well with the in vitro digestion protocol (Beer et al 1997b) and it did not require any pre-digestion or sample extraction steps.

The molecular weight of soluble β-glucan in extracts is typically analysed by high performance size exclusion chromatography with online post column detection with Calcofluor, which is widely used for characterising β-glucan molecules (Wood et al 1991b; Suortti 1993). The molecular weight distribution of β-glucan is calculated by comparison to standards of known molecular size. This is a useful procedure as the β-glucan can be detected and characterised even in the presence of other polysaccharides in the extract (Wood et al 1991b; Rimsten et al 2003; Wang and Ellis 2014).

In literature, most studies investigated the differences in β-glucan viscosity among different oat incorporated food products due to the impact of processing and formulation on β-glucan physicochemical properties such as molecular weight and solubility (Tosh et al 2010; Gamel et al 2012; Gamel et al 2014). Limited research has been conducted that show a variation in viscosity and molecular weight of extracted β-glucan from oats, due to the impact of cultivar and environment (Colleoni- Sirghie et al 2003; Andersson and Börjesdotter 2011). Colleoni- Sirghie et al 2003 studied and compared the apparent viscosity of extracted β-glucan from 6 oat cultivars at shear rate of 50 sec^{-1} using a rotational rheometer. The extracted β-glucan from each oat cultivar was studied at different concentrations. MW, genotype and β-glucan concentration affected the value of apparent viscosity of the solution. However the rate of change of viscosity varied amongst cultivars. In another study by Andersson and Börjesdotter (2011) it was noted that 4 oat cultivars grown over 11 different environments had variation in β-glucan content and molecular weight. Environment had higher impact on β-glucan molecular weight and genotype had a greater impact on β-glucan content. Therefore, more research is required to understand the variation in oat β-glucan physicochemical properties due to the impact of genotype, environment and processing. Therefore, the aim of this research was to investigate the viscosity, β-glucan content, solubility and molecular weight of β-glucan extracted under physiological conditions, in different oat cultivars over two growing seasons as milled oat flour and after processing into 30% oat-wheat white salted noodles (30% OW noodles).

6.2 Summary of Materials and Methods

6.2.1 Materials

Six milling oat cultivars (Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams) grown over two different seasons (2011 and 2012) at the same location (Katanning, WA) were selected for this study. Oat cultivars were supplied by the National Oat Breeding Program managed by the South Australian Research and Development Institute (SARDI), in collaboration with the Department of Agriculture and Food Western Australia (DAFWA). All tests were done at least in duplicate. The wheat flour (control) was commercial udon flour from Japan.

6.2.2 Samples

Oat grains were processed to oat flour (Refer to *section 3.2. Sample preparation* for further details). The official Japanese noodle processing steps (AEGIC) were used to prepare the white salted noodles (WSN) with some modifications for 30% OW noodles (Mitra et al 2012) (Refer to *section 3.4.2; Table 3.3 Steps involved in WSN udon noodle processing* for further details).

These noodles were cooked based on their optimum cooking time (OCT) (Approved Method, 66-50.01 AACCI 2010) and the cooked 30% OW noodles (30% OW noodles) and control wheat noodles (Refer to *section 3.4.3 cooking of.30% oat-wheat noodles and control wheat noodles* for further details) were then freeze dried using a freeze dryer (Alpha 1-2 LD Plus, John Morris Scientific, Sydney, Australia) and ground to a powder using a coffee grinder for 1 min (Breville; Model No. BCG300).

6.2.3 Measurement of β-glucan viscosity

The Gamel et al (2012; 2014) method was used to determine the viscosity of β-glucan using the Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia. Some modifications were made to this method, for both the 100% oat flour and 30% OW noodle samples tested (Refer to *section 3.5 Measurement of β-glucan viscosity* for further details). Pre-cooked oat flour samples and freeze dried 30% OW noodle samples were mixed with the sodium phosphate buffer (pH 6.9) in the RVA canisters and to it the enzyme combination of microbial α-amylase (30 U) and microbial protease (20 U) was added. The final viscosity of the flour slurry from oat flour and 30% OW noodle samples was recorded at the end of the RVA run time (2 h). After enzyme treatment, the slurry samples (mixture of the sample, buffer and enzymes) of oat flour/30% OW noodle were centrifuged at 8000 x g at 20°C for 10 min. The supernatant, containing the extracted soluble β-glucan, was analysed for viscosity, β-glucan content, β-glucan solubility and β-glucan molecular weight (Refer to *section 3.5 Measurement of β-glucan viscosity* for further details).

6.2.4 Supernatant viscosity

The viscosity of the supernatant, containing β-glucan, was measured using a viscometer (Brookfield, DV-I + viscometer) with enhanced UL adapter kit (Brookfield Engineering Labs. Inc., Stroughton, USA) with ULA spindle (shear rate 122 sec^{-1}) to measure low viscosity materials (Turnbull et al 2005), like 30% OW noodle extract and small sample adapter kit (Brookfield Engineering Labs. Inc., Stroughton, USA)

with cylindrical spindle SC4-29 spindle (shear rate 25 sec^{-1}) to measure viscosity of 100% oat flour extract with high viscosity (Refer to *section 3.5 Measurement of β-glucan viscosity*for further details).

6.2.5 β-glucan content of 100% oat flour and 30% OW noodle supernatant

The (1-3)(1-4)-β glucan content in the control wheat flour and the oat flour samples was determined using a mixed-linkage β-glucan kit assay (Megazyme International Ltd., Wicklow, Ireland) using Approved Method 32-23.01 (AACC International 2010) (Refer to *section 3.3.2Oat flour quality*for further details).

The β-glucan content of freeze dried cooked noodles (control and 30% OW noodles) and freeze dried supernatants from 100% oat flour and 30% OW noodles were analysed using the mixed-linkage β-glucan kit (Megazyme International Ltd., Wicklow, Ireland) using the standard procedure for cooked, toasted or extruded cereal products based on Approved Method 32-23.01 (AACC International 2010) (Refer to *section* 3.4.4.1 *β-glucan content of cooked* noodles and sectio*n* 3.5.3.2 *β-glucan content of the extract* further details).

6.2.6 β-glucan solubility

The percent extractable (solubilised) β-glucan at 37°C was calculated using the following formula (Tosh et al 2010): Percentage soluble β-glucan (%) = (soluble β-glucan/total β-glucan) x 100. The β-glucan content solubilised in the supernatant and the total β-glucan content of 100% oat flour and 30% OW noodles were used in the calculation.

6.2.7 Peak molecular weight of β-glucan

The peak molecular weight (M_p) and average molecular weight (M_w) of the extracted β-glucan from 100% oat flour and 30% OW noodle samples, was measured using high performance size exclusion chromatography- HPSEC (Agilent 1100/1200 HPLC) with post column addition of fluorescent brightener (FB) 28 (Calcofluor) and fluorescence detection (Shimadzu fluorescence detector RF-10AXL) (Refer to *section* 3.5.3.4 *Molecular weight of β-glucan* for further details).

6.3 Statistical Analysis

Data was analysed using SPSS Version 21.0 (SPSS, Chicago, IL). The effects of cultivars; years; and the interaction between cultivar by years; was assessed using

Analysis of Variance (ANOVA). Post-hoc comparison of means for significant effects within the Analysis of Variance was carried out using Least Significant Difference (LSD). The association between RVA final viscosity/Log (RVA final viscosity) and other viscosity parameters/Log (M_p X C) or Log (M_w X C) were assessed using Pearson's correlation coefficient and linear regression. *P*-values less than 0.05 were considered as statistically significant.

6.4 Results and Discussion

6.4.1 Viscosity curve

Figure 6.1 (a,b) and 6.2 (a, b) represent the RVA viscosity curves of oat flour samples and 30% OW noodle samples, respectively, developed over a period of 2 h with a combination of digestive enzymes. Since, 100% oat flour had significantly ($P<0.05$) higher content of β-glucan (4.59 - 5.92%) in comparison to the 30% OW noodles (1.24 - 1.66%), the amount of sample used for generating the β-glucan viscosity curve was 2 g (dwb) for 100% oat flour and 4.5 g, (dwb) for 30% OW noodle (ground samples) in order to obtain meaningful RVA results.

RVA viscosity curve of oat flour (Figure 6.1 a, b) and 30% OW noodles (Figure 6.2 a, b) differed between the oat cultivars within a year and between the same cultivars over two different years. The difference in viscosity profile between oat cultivars is largely due to their variation in β-glucan content, β-glucan solubility and β-glucan molecular weight for oat flour and 30% OW noodles as presented in Table 6.1 and 6.2. The food matrix and composition of the oat flour and 30% OW noodle samples would also have a high impact on the shape of the viscosity curve.

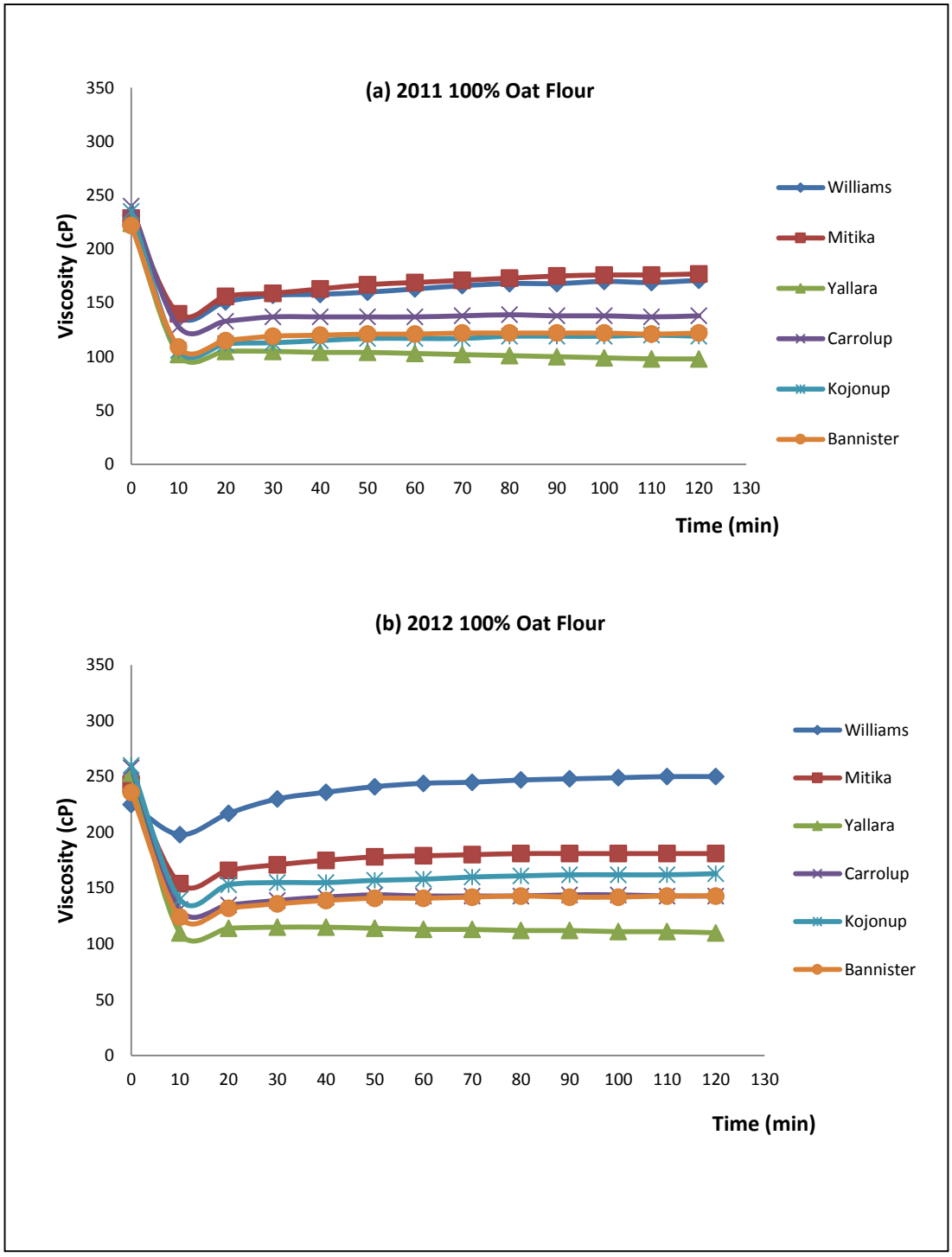

Figure 6.1 RVA viscosity curve of 100% oat flour treated with 30 U microbial αamylase and 20 U microbial protease

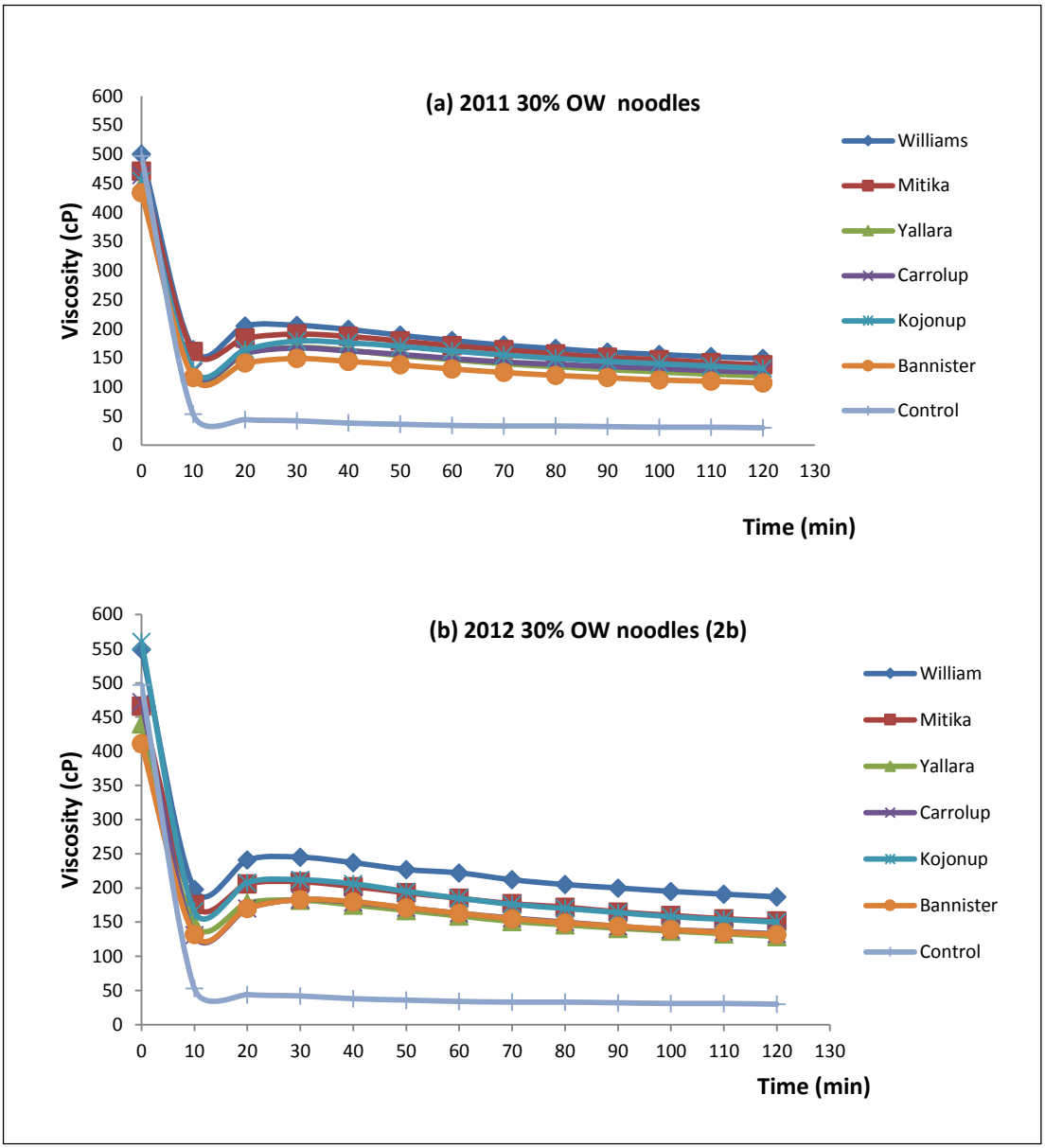

Figure 6.2 RVA viscosity curve of 30% OW noodles treated with 30 U microbial α-amylase and 20 U microbial protease

According to Gamel et al (2012), it is important for the viscosity curves to plateau between 1 and 2 h of the RVA run time as this will indicate digestion of food components like starch and protein due to the digestive enzymes and therefore limit the impact of these components on sample viscosity. As seen in Figure 6.1 and 6.2, the oat flour and 30% OW noodle samples RVA viscosity curve plateaued between 1 and 2 h indicating the final viscosity recorded at the end of the RVA run time could be correlated to the β-glucan physicochemical properties without much interference of other food components. Although there could have been some undigested starch and protein which could have also contributed to some of the viscosity. However, the addition of lichenase along with digestive enzymes showed an extensive reduction of final viscosity. After addition of lichenase with the digestive enzymes, Williams (2012) oat flour which had the highest final viscosity it reduced from 249 to 16 cP and for 30% OW noodles incorporated with Williams, the final viscosity reached to the same level as the control sample and it reduced from 187 to 30 cP. This shows that the contribution to the viscosity for these curves without lichenase enzyme was mainly due to β glucan.

6.4.2 Effect of cultivar and environment on physicochemical properties of β-glucan for oat flour and 30% OW noodles

The physicochemical properties (β-glucan content of the samples, β-glucan content of the supernatant, final viscosity, solubility of β-glucan at 37°C, and peak and average molecular weight of the solubilised β-glucan in the supernatant) are shown in Table 6.1 for oat flour samples and Table 6.2 for 30% OW noodles.

The effect of year of cultivation was significant (P<0.05) for final viscosity, β-glucan content of the supernatant and the solubility percentage of the β glucan, for both oat flour and 30% OW noodles. The peak and average molecular weight of the β-glucan in supernatant was also found to be significant (P<0.05) between years for oat flour. The effect of year was not significant (P>0.05) for peak and average molecular weight of the β-glucan in supernatant from 30% OW noodles and β-glucan content of oat flour and 30% OW noodles. The effect of cultivar was significant for the all viscosity parameters. The interaction between cultivar and year of cultivation was significant (P<0.05) for all the viscosity parameters except for the peak and average molecular weight of β-glucan in supernatant from oat flour and β-glucan content of the supernatant from 30% OW noodles.

6.4.3 Final viscosity and its association with viscosity parameters

The viscous properties of a β-glucan solution are known to be influenced by different factors such as the concentration, solubility and molecular weight of the extracted β-glucan (Beer et al 1997b; Rimsten et al 2003; Ajithkumar et al 2005; Wolever et al 2010). The RVA final viscosity of oat flour samples and 30% OW noodles after 2 h is presented in Table 6.1 and 6.2. The final viscosity of 100% oat flour and 30% OW noodles in 2012 of each oat cultivar was equal or higher than the same cultivar grown in 2011.

Williams and Mitika oat flour had the highest final viscosity and Yallara had the lowest final viscosity for both the years for oat flour samples (Table 6.1). The final viscosity of oat flour ranged from 97 to 249 cP for oat flour samples. For the 30% OW noodles, Williams had the highest final viscosity and Carrolup, Yallara and Bannister had the lowest final viscosity for both years (Table 6.2). The final viscosity for 30% OW noodles ranged from 105 to 187 cP. In addition, the final viscosity of the control wheat noodle, 30 cP, was lower than the 30% OW noodles as the β-glucan content of the wheat noodle was almost negligible (β-glucan content 0.27%). A very strong positive correlation was noted between RVA final viscosity and β-glucan content of the oat flour (r = 0.909 for 2011, P<0.05 and r = 0.931 for 2012, P< 0.01) and 30% OW noodles (r = 0.939 for 2011, P <0.05; r = 0.98 for 2012, P< 0.01). Earlier studies on oat lines and oat products have shown that samples with higher β-glucan content, extracted β-glucan with higher viscosity (Gamel et al 2012; Colleoni-Sirghie et al 2003). However, the percentage of β-glucan that is solubilised at 37°C provides the viscosity to the extract. Thus, β-glucan content of oat flour

Table 6.1 Physicochemical characteristics of 100% oat flour

Cultivars	β-glucan content (%) of oat flour^		Final Viscosity (RVA) ^ (cP)[x]		β-glucan content (%) [XX] of supernatant ^		Solubility (%) of β-glucan^ At (37° C) [y]		M_p of extracted β-glucan# (kDa)[XX]		M_w of extracted β-glucan# (kDa)[XX]	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Mitika	5.38b ±0.08	4.93c* ±0.11	180a ±4.24	180b ±2.12	3.94a ±0.01	3.93b ±0.01	36.1a ±0.64	39.7a* ±0.93	2740 ±23.28	2821 ±6.97	1900 ±37.53	1979 ±6.40
Kojonup	4.90c ±0.00	5.31b* ±0.01	121c ±2.12	162c* ±1.41	3.07d ±0.05	3.49c* ±0.02	32.5cd ±0.52	33.7c ±0.25	2703 ±10.65	2807 ±3.90	1889 ±9.73	1992 ±11.25
Yallara	4.59e ±0.02	4.56e ±0.04	97d ±0.00	113e* ±4.24	2.87e ±0.04	2.99e* ±0.01	31.3d ±0.61	36.4b* ±0.25	2685 ±21.41	2702 ±2.39	1848 ±1.13	1870 ±18.97
Bannister	4.76d ±0.08	4.79cd ±0.04	118c ±5.66	148d* ±6.36	3.00d ±0.14	3.41cd* ±0.02	33.4bc ±1.03	36.5b* ±0.5	2647 ±20.05	2763 ±60.25	1842 ±15.64	1939 ±33.47
Carrolup	5.00c ±0.14	4.74d* ±0.03	134b ±5.66	140d ±6.36	3.22c ±0.01	3.32d* ±0.01	33.9b ±1.03	35.6b* ±0.36	2722 ±41.85	2784 ±8.67	1980 ±64.28	1963 ±19.99
Williams	5.92a ±0.01	5.93a ±0.00	174a ±5.66	249a* ±1.41	3.64b ±0.01	4.15a* ±0.01	32.3cd ±0.14	35.3b* ±0.06	2718 ±31.99	2785 ±86.30	1914 ±17.77	1986 ±29.21

[x] As measured by RVA before centrifugation; [XX] Freeze dried supernatant; [y] In relation to total β-glucan; ^ Year and cultivar interaction was significant ($P<0.05$); # Year and cultivar interaction was not significant ($P>0.05$); a Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);*Notes the significant difference in mean values between different years ($P<0.05$)

Table 6.2 Physicochemical characteristics of 30%OW noodle

Cultivars	β-glucan content (%) of 30 % OW noodles ^		Final Viscosity (RVA) ^ (cP) [x]		β-gluca content (%) [XX] of supernatant #		Solubility (%)of β-glucan ^ At (37° C) [y]		M_p of extracted β-glucan ^ (kDa) [XX]		M_w of extracted β-glucan ^ (kDa) [XX]	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Mitika	1.52b ±0.04	1.43c* ±0.01	140b ±2.83	151b* ±1.41	1.18 ±0.01	1.24 ±0.01	30.8b ±0.81	35.1a* ±0.34	1102b ±24.68	1058c* ±3.38	1199b ±16.16	1140b* ±5.84
Kojonup	1.42c ±0.04	1.5b* ±0.00	132c ±0.71	153b* ±4.24	0.98 ±0.01	1.17 ±0.01	29.1b ±1.00	33.6abc* ±0.44	1165a ±10.52	1166a ±2.32	1271a ±16.74	1266a ±0.68
Yallara	1.36d ±0.02	1.36d ±0.06	122d ±0.424	129c ±0	0.92 ±0.001	1.07 ±0.02	29.2b ±0.43	32.3c* ±1.88	1102b ±3.37	1019e* ±2.75	1185b ±8.76	1059c* ±5.84
Bannister	1.24e ±0.01	1.37d* ±0.05	105e ±2.83	131c* ±1.41	1.00 ±0.002	1.12 ±0.21	33.1a ±0.28	35.0ab* ±1.94	1018d ±8.34	1029de ±0.87	1027d ±8.18	1071c* ±5.37
Carrolup	1.45c ±0.01	1.38cd* ±0.07	120d ±9.19	131c* ±3.53	1.08 ±0.02	1.11 ±0.003	32.8a ±0.86	33.0bc ±1.63	1054c ±7.62	1046cd ±14.98	1106c ±11.69	1115b ±2.72
Williams	1.66a ±0.03	1.65a ±0.03	150a ±0.71	187a* ±0.00	1.24 ±0.05	1.37 ±0.02	25.2c ±0.61	28.9d* ±0.80	1062c ±2.42	1144b* ±10.50	1122c ±10.55	1286a* ±14.86

[x] As measured by RVA before centrifugation; [XX] Freeze dried supernatant ; [y] In relation to total β-glucan; ^ Year and cultivar interaction was significant (P<0.05); # Year and cultivar interaction was not significant (P>0.05); a Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05);*Notes the significant difference in mean values between different years (P<0.05).

and 30% OW noodle samples along with the β-glucan solubility (%) at 37°C was assessed in this study.

Although the effect of year on β-glucan content of oat flour and 30% OW noodles was not significant ($P>0.05$), the effect of year on β-glucan solubility at 37°C was significant ($P<0.05$). The β-glucan solubility of oat flour (Table 6.1) and 30% OW noodles (Table 6.2) for each oat cultivar grown in 2012 was higher than in 2011. For 100% oat flour samples, the solubility of β-glucan ranged from 31.3-36.1% in 2011 and 33.7-39.7% in 2012. For 30% OW noodle samples the solubility ranged from 25.2-33.1% for 2011 and 28.9- 35.1% for 2012. Beer et al (1997b) reported that 12-33% of total β-glucan in bran and rolled oats are soluble although Tosh et al (2010) gave a slightly higher value of 39% for solubility of commercial oat bran. The higher β-glucan solubility for 2012 may have resulted in higher extraction of β-glucan of each oat cultivar grown in 2012 in comparison to the same cultivar grown in 2011 for 100% oat flour and 30% OW noodle with the exception of Mitika oat flour sample.

For oat flour samples (Table 6.1), Williams had the highest β-glucan content (5.92% in 2011 and 5.93% in 2012) which may have resulted in a high final viscosity (174 cP for 2011 and 249 cP for 2012) and Yallara had the lowest β-glucan content (4.59% for 2011 and 4.56% for 2012) which may have resulted in lowest final viscosity (97 cP for 2011 and 113 cP for 2012) for both the years. Mitika (5.38%) had the second highest β-glucan content to Williams for 2011 and its β-glucan content was lower than Kojonup in 2012 (5.31%). However, Mitika had highest solubility percentage (36.05% for 2011 and 39.7% for 2012) and high extract β-glucan content (3.94% in 2011). Therefore the viscosity profile and final viscosity of Mitika in 2011(180 cP) was highest and not significantly ($P>0.05$) different to Williams 2011 (174 cP). For 30% OW noodles samples (Table 6.2), Williams had the highest β-glucan content (1.66% in 2011 and 1.65% in 2012) and highest final viscosity (150 cP for 2011 and 187 cP for 2012) and Yallara and Banister had the lowest β-glucan content (1.36% for 2011; 1.36% for 2012 and 1.24% for 2011 and 1.37% for 2012, respectively) and had lowest final viscosity (122 cP for 2011; 129 cP for 2012 and 105 cP for 2011 and 131 cP for 2012, respectively).

The RVA final viscosity of oat flour and 30% OW noodles might have been influenced by the particulate matter present in them. Therefore to remove the interference of

particulate matter the flour slurry was centrifuged, at the end of enzymatic digestion in RVA, and the supernatant viscosity was measured using a viscometer as suggested by Gamel et al (2012; 2014). The supernatant viscosity (Refer to *Appendix 7; Table 7A and 7B* for further details) after centrifugation correlated very strongly with the final viscosity before centrifugation, from oat flour (r = 0.995, $P<0.001$ for 2011 and r = 0.990 for 2012, $P<0.001$) and 30% OW noodles (r = 0.908 for 2011, $P<0.05$ and r = 0.98, $P<0.01$), showing that the particulate matter did not influence the viscosity ranking of the cultivars for oat flour and 30% OW noodle samples. Similar findings were reported by Gamel et al (2012; 2014). In this study the centrifuged supernatant of the extracted β-glucan was then freeze dried and the supernatant (extract) β-glucan content and molecular weight were analysed and correlated with the RVA final viscosity.

The freeze dried extract's (supernatant) β-glucan content for oat flour (Table 6.1) ranged from 2.87 -3.94% for 2011 and 2.99-4.15% for 2012 and for 30% OW noodles ranged from 0.92-1.24% for 2011 and 1.07-1.37% for 2012. For extract β-glucan content from oat flour, Williams and Mitika had the highest value and Yallara had the lowest value for both the years. For 30% OW noodles, extract β-glucan content, the interaction of cultivar and year was not significant ($P<0.05$). The average β-glucan content of the supernatant from 30% OW noodles for an oat cultivar over 2 years (Refer to *Appendix 7; Table 7C* for further details) showed that Williams (1.30%) had significantly highest extract β-glucan content ($P<0.05$), followed by Mitika (1.21%) and lowest for Yallara (0.99%). A strong positive correlation was seen between final viscosity and supernatant β-glucan content for oat flour ($r = 0.979$ for 2011, $P<0.001$ and $r = 0.946$, $P<0.01$ for 2012) and for 30% OW noodles the correlation was strong for 2012 ($r = 0.966$ for 2012 at $P<0.01$) and strong but non-significant for 2011 ($r = 0.723$ for 2011 at $P> 0.05$). The non-significant correlation between final viscosity and supernatant β-glucan content of 30% OW noodles for 2011, can be associated to the fact that the supernatant β-glucan content of 30% OW noodles for Bannister, 2011(1.00%) was higher than 30% OW noodles Yallara 2011(0.92%) and 30% OW noodles Kojonup, 2011 (0.98%). However 30% OW noodles for Bannister (2011) still had lowest final viscosity, which could be because it had significantly lowest ($P< 0.05$) molecular weight (M_p and M_w) (Table 6.2) in comparison to other oat cultivars incorporated in 30% OW noodles.

Viscosity of β-glucan in a solution depends on both, concentration (C) and molecular weight of β glucan (Tosh et al 2008). The association between log (final viscosity) and log (M_p x C) for oat flour (r = 0.968, P< 0.01 for 2011 and r = 0.945, P< 0.01 for 2012) and 30% OW noodles (r = 0.892, P<0.05 for 2011 and r = 0.982, P< 0.001, for 2012); and the association between log (final viscosity) and log (M_w x C) for oat flour (r = 0.974, P< 0.01 for 2011 and r = 0.961, P< 0.01 for 2012) and 30% OW noodles (r = 0.837, P< 0.05 for 2011 and r = 0.984, P<0.001 for 2012) was linear and very strong, as predicted by polymer theory in other studies (Wood et al 2000; Tosh et al 2010).

6.4.4 Impact of processing on molecular weight

The molecular weights (M_p and M_w) of β-glucan extracts from oat flour and 30% OW noodles fall above the calibrated molecular weight range. The M_p and M_w are therefore calculated values which might denote only an estimated peak or average molecular weight of the samples and not the actual peak or average molecular weight.

The chromatograms of β-glucan molecular weight for both oat flour and 30% OW noodles show a bimodal distribution of for both years (Figure 6.3 and 6.4). Peak (M_p) and average (M_w) molecular weight of the extracted β-glucan at 37°C from 100% oat flour was significantly (P<0.05) higher than the 30% OW noodles (Table 6.1 and 6.2). The peak molecular weight (M_p) of extracted β-glucan from oat flour ranged from 2821-2647 kDa and the average molecular weight (M_w) ranged from 1842-1992 kDa. In the literature, a wide range of molecular weight (1500 – 3000 kDa) has been reported for oat β-glucan (Wood 1991; Autio et al 1992; Beer et al 1997a; Wang and Ellis 2014). This variation could be due to different methodologies used for extraction and characterisation of oat β-glucan. The peak molecular weight (M_p) of β-glucan from oat flour samples in this study was consistent with the molecular weights reported by Wood et al (1991b) (2500 kDa); Beer et al (1997a) (2000- 2500 kDa); Wang and Ellis 2014 (2000 – 3000 kDa) and Colleoni-Sirghie et al (2003) (≥ 2000 kDa). Although statistically significant differences in the molecular weight between certain oat cultivars were noted in this study, the variation between cultivars however was small, as noted by other studies (Beer et al 1997a; Colleoni-Sirghie et al 2003). For oat flour samples, the M_p (average of a cultivar over 2 years) of the extracted β-glucan (Refer to *Appendix 7; Table 7D* for further details) of Mitika (2781 kDa) was highest and not significantly (P> 0.05) different to Kojonup (2755 kDa), Carrolup (2753 kDa) and Williams (2752 kDa), but significantly higher (P<0.05) than Yallara (2693 kDa), and

Bannister (2705 kDa). For the M_w (average of a cultivar over 2 years) of oat flour samples, Carrolup (1972 kDa) was highest and not significantly (P>0.05) different to Mitika (1939 kDa), Kojonup (1941 kDa) and Williams (1950 kDa), but significantly higher (P<0.05) than Yallara (1859 kDa) and Bannister (1891 kDa).

Molecular weight of β-glucan is known to decrease after being processed into food products for example in bread making, baking of muffins or cookies, and fresh pasta. (Beer et al 1997b; Åman et al 2004; Andersson et al 2004). The peak and average molecular weight of β-glucan extracted from 30% OW noodle samples was much lower than the molecular weight of β-glucan extracted from the oat flour samples for all oat cultivars. The peak molecular weight and average molecular weight of β-glucan extracted from 30% OW noodles ranged from 1018-1144 kDa and 1027-1286 kDa, respectively. For 30% OW noodle samples (Table 6.2), Kojonup (1165 kDa for 2011 and 1166 kDa for 2012) had the highest (P<0.05) M_p and Kojonup (1271 kDa for 2011 and 1266 kDa for 2012) and Williams for 2012 (1286 kDa) had the highest M_w.Whereas, Banister for 2011 (M_p -1018 kDa; M_w 1027 kDa) and Yallara for 2012 (M_p-1029 kDa; M_w-1071 kDa) had the lowest M_p and M_w. The control wheat noodle sample had a peak molecular weight of 33.6 kDa. Thus, it can be noted that noodle processing had a significant effect on the β-glucan molecular weight of oat cultivars.

However, the percent decrease in molecular weight (Refer to *Appendix 7; Table 7E and 7F* for further details) of β-glucan from oat flour to processed 30% OW noodles varied between cultivars and ranged from 57- 63% for peak molecular weight and ranged from 33-45% for average molecular weight. The percent decrease in β-glucan M_p and M_w after processing into 30% OW noodles was significantly lower for Kojonup (M_p: 57% for 2011; 58% for 2012 and M_w: 33% for 2011; 36% for 2012) for both the years and Williams (M_p: 59% and M_w: 35%) for 2012, in comparison to the other oat cultivars. This significant reduction in the M_p and M_w of β-glucan from oat flour to processed 30% OW noodles is due to the impact of processing; however

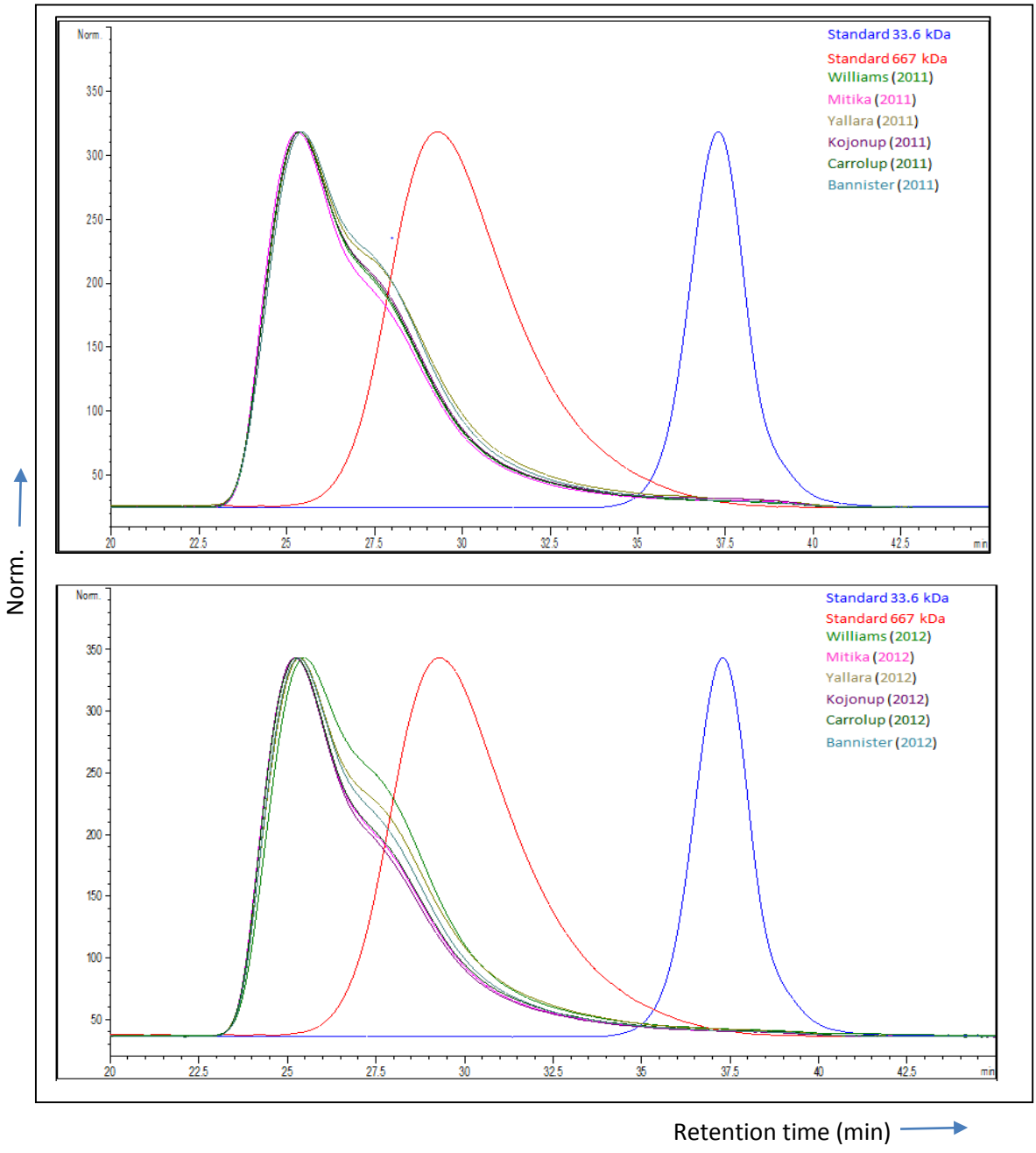

Figure 6.3 SEC-FD Chromatograms of soluble (1,3);(1,4)-β-glucan extracted from 100% oat flour (2011 and 2012)

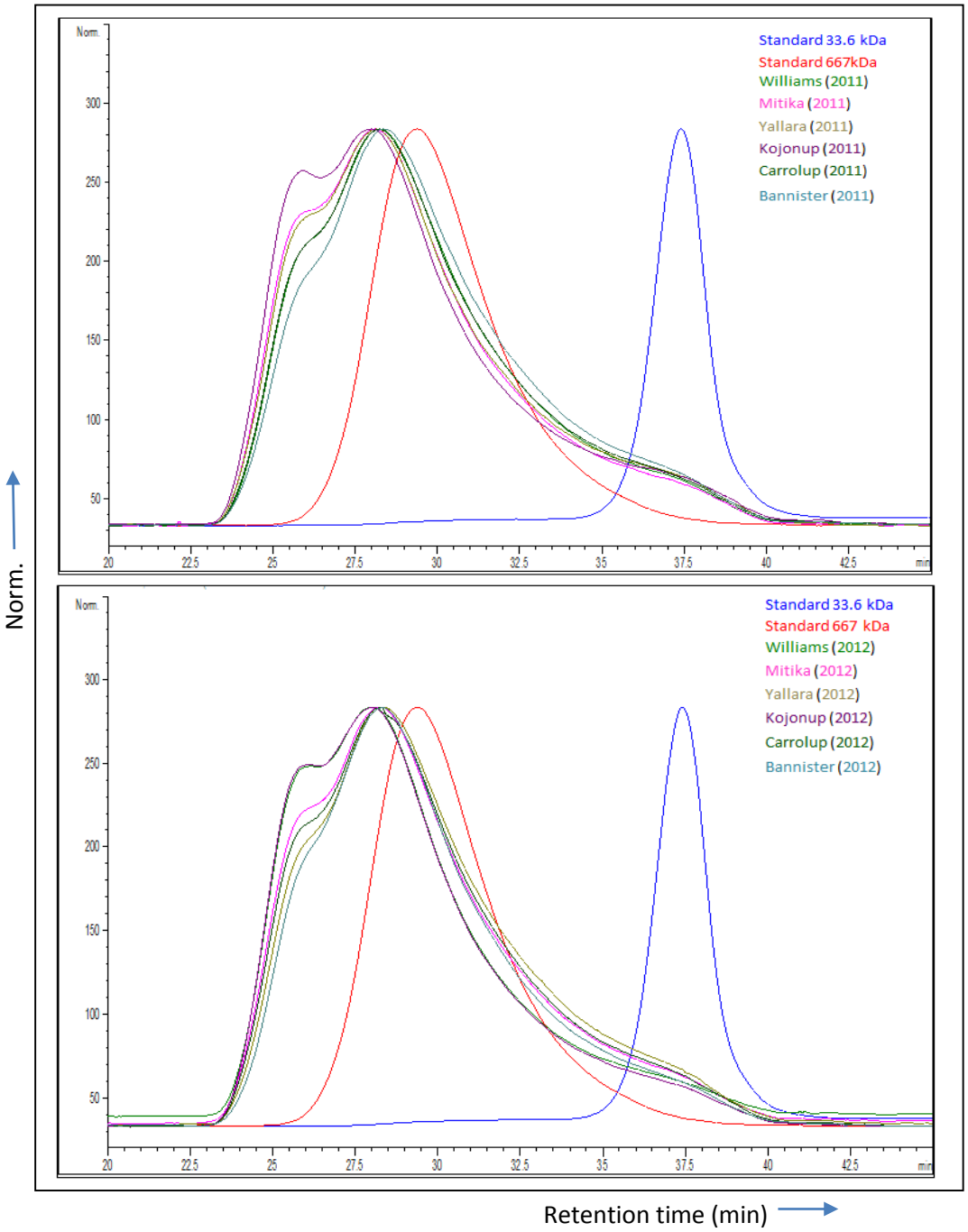

Figure 6.4 SEC-FD Chromatograms of soluble (1,3);(1,4)-β-glucan extracted from 30% OW noodles (2011 and 2012)

Identification of the processing stages or conditions which have the greatest effect on β-glucan molecular weight is more challenging.

Depolymerisation of β-glucan during processing of some foods has been reported in literature (Åman et al 2004; Tosh et al 2010). Changes in the properties of β-glucan during processing can arise from shearing damage due to mechanical processing (Wood et al 1989), or by excessive heat treatment of food products (Brennan and Cleary 2005). Wheat flour contains active β-glucanase enzymes which contribute to the degradation of β-glucan in oat incorporated wheat based products (Åman et al 2004; Andersson et al 2004; Andersson et al 2008). Therefore, the processing steps to make 30% OW noodles, which includes mixing, sheeting, cutting, resting of the noodle sheets, and boiling of noodles, together with the β-glucanase enzymes present in the wheat flour, may have each contributed to the reduction in molecular weight of the β-glucan extracted from 30% OW noodles. However, it is expected that the molecular weight of the β-glucan after processing would still be high enough to promote health benefts, although clinical studies using these samples would be needed to confirm this.

Furthermore, a slight reduction in the solubility of β-glucan at 37°C was also noted from oat flour to 30% OW noodles. Similar to molecular weight, this change in solubility is thought to be due to the effects of processing oat flour into noodles. In other research work contradictory results were noted where the oat bran was incorporated in extruded cereals and the solubility of β-glucan increased markedly (Tosh et al 2010). According to a study conducted by Tosh et al (2008), an initial decrease in molecular weight of β-glucan during processing increased the solubility of β-glucan. However, a further subsequent reduction in β-glucan molecular weight decreased its solubility due to stronger self-association of the depolymerised β-glucan and insoluble aggregates are formed. Lower molecular weight polysaccharides have increased reactivity with other components of food contributing to low solubility (Tosh et al 2004). Frozen storage of oat containing products also affects β-glucan solubility by reorganisation of β-glucan chains due to intermolecular interactions and this leads to an ordered structure (Beer et al 1997b; Lazaridou and Biliaderis 2004). Therefore, the depolymerisation of β-glucan in oat cultivars after noodle processing, reaction of the low molecular weight β-glucan with other components of 30% OW noodle blends, freeze drying of 30% OW cooked noodle samples and its subsequent storage in the

freezer could have contributed in the reduced solubility of β-glucan from 30% OW noodles in comparison to the β-glucan solubility of oat flour.

6.5 Conclusion

The Australian milling oat β-glucan viscosity varied between different oat cultivars and growing seasons in flour and 30% OW noodles. β-glucan final viscosity was found to be significantly correlated to a number of oat flour and noodle properties including its β-glucan content, extract β-glucan content and combined effect of β-glucan molecular weight with extract concentration of β-glucan. The processing conditions involved in the manufacture of noodles had an impact on the oat β-glucan specifically the β-glucan solubility and extracted β-glucan molecular weight; reducing the peak molecular weight by 60% and average molecular weight by 40%. In all oat cultivars the extracted β-glucan from 30% OW noodles had lower solubility and significantly lower (P<0.05) peak and average molecular weight (depolymerisation) over the two growing seasons in comparison to the extracted β-glucan from oat flour. This study provides evidence that physicochemical properties of β-glucan in oat flour or oat-wheat noodles play a crucial role in development of its viscosity in a solution at 37°C. Williams and Mitika have been identified as the oat cultivars which had the highest extracted β-glucan final viscosities from oat flour and 30% OW noodle samples for both years. Yallara oat flour and Yallara incorporated 30% OW noodles, and Bannister incorporated 30% OW noodles (2011), were identified as the cultivars with the lowest β-glucan final viscosities. β-glucan extracted from Kojonup oat flour and Kojonup incorporated 30% OW noodles were most resistant to molecular weight breakdown due to processing.

This study shows that analysing the β-glucan content of an oat cultivar is not sufficient characterisation for selecting oat cultivars with significant health benefits because although the milling oat cultivars analysed in this study varied by only 1% or less in β-glucan content, they showed a wide range in extracted β-glucan viscosity. Therefore, it is also important to understand other properties of oat β-glucan such as its viscosity, solubility and molecular weight in the gut environment, pre and post processing. Since the β-glucan extraction in this study was carried out at body temperature of 37°C with digestive enzymes, the viscosity developed by the β-glucan can be related to its expected health benefits or bioactivity. However, to directly correlate these physiological properties of β-glucan to its physiological effectiveness, in vivo studies

with these oat cultivars will need to be carried out in the future. Results obtained from those future human studies would facilitate the understanding and help us to interpret if the β-glucan viscosity differences between these oat cultivars noted in this study actually have any significant physiological differences in providing health benefits.

Chapter 7: Conclusion and Future Directions

This research study involved the selection and milling of six oat cultivars from W.A. (Mitika, Kojonup, Carrolup, Yallara, Bannister and Williams) which were grown over two growing seasons (2011 and 2012) at the same location (Katanning).

The characterisation of the flour from different oat cultivars over two growing season in terms of its physicochemical properties such as pasting properties, β-glucan content, proximate analysis, starch composition, particle size and colour were determined. The findings of this study show that genotype and environment are important factors that affect oat grain quality including composition, pasting properties and colour. The growing year 2012 had moderate rainfall of 481.0 mm compared to 2011 which had higher rainfall of 668.4 mm. Rainfall and other environmental differences in the growing years could have been the reason for differences in composition, colour and particle size. The 2012 oat cultivars having higher average values (average across six cultivars grown in a year) for protein content, lipid content and lower average values (average across six cultivars grown in a year) for ash content, and starch damage. Brightness/whiteness (CIE *L**) was generally higher in 2012, and yellowness (CIE *b**) was higher and redness (CIE *a**) lower in 2012. The colour stability of 30% oat-wheat raw noodle sheets, showed on average that cultivars grown in 2012 had better colour stability than cultivars grown in 2011. Milling produced a greater volume of finer particle size flour from cultivars grown in 2012 with up to 61.6% of particles in the <100 μm range and therefore a lower volume of coarser particle size flour(>100 μm) in comparison to the same cultivars grown in 2011.

There was an effect of genotype on colour and oat cultivar Yallara had superior colour quality in terms of groat brightness, flour brightness and this brightness was found in both small and large flour fractions (<100 μm and >100 μm). This groat and flour brightness translated to noodle brightness. Mitika was identified as having the highest peak viscosity, amylopectin content, protein content and greater percentage of finer particle size flour.

This research supports and adds to other research on how environment and cultivar can have an impact on the physicochemical properties of Australian oats but in future it is important to investigate the influence of different environmental parameters in

addition to rainfall such as soil, climate and agronomy on quality of oat cultivars. Also, in this study oat cultivars were selected from only one location and for future research similar cultivars from different locations should be examined.

The effect of incorporating oat flour from different oat cultivars from two growing seasons on quality of white salted noodles (WSN) provided valuable information. Based on preliminary work, 30% of oat flour was incorporated in wheat noodle formulation for producing 30% OW noodles and compared with control wheat flour/ white salted noodles. 30% OW noodles with the incorporation of all six oat cultivars from two growing seasons were successfully produced. However, they were significantly different to control wheat (udon) flour/noodles, as expected, with wheat (udon) noodles having superior quality with brighter colour, firmer texture, lower solid loss and higher colour stability. However, this research shows that oat flour is superior in terms of β-glucan content and protein content in comparison to wheat (udon) flour. Flour quality parameters of oat cultivars from two growing seasons showed a good correlation to the 30% OW blend and 30% OW noodle quality measured, so flour quality could be used to predict noodle quality.

The seasonal impact was stronger than genotype effect on texture of 30% OW noodles firmness. However, for colour values and colour stability of 30% OW noodles, the 2012 oat cultivars were superior to the same cultivar grown in 2011; possible reasons could be the higher rainfall in 2011, which caused discolouration in oat grains but may be due to variation in enzymatic or non-enzymatic browning during noodle processing. Higher percentage of coarser particle size flour, as noted for oat cultivar Williams, may have negatively impacted 30% OW noodle quality such as solid loss and β-glucan loss in cooking water and produced noodles which were dull with higher specks. Among all the oat cultivars Mitika was easiest to process and was identified as the most suitable cultivar for incorporation in WSN noodles for each year, due to its pasting properties, noodle colour, colour stability, β-glucan content (which was moderately high) and stability. This study gives valuable information on quality of oat-wheat white salted noodles and how the quality varies due to season and cultivar, which was lacking in the literature. In the future more investigation is needed to standardise the quality parameters required for producing the best quality oat-wheat noodles by selecting the same or different cultivars from different growing locations and environments. Sensory evaluation with a trained and consumer panel should also

be conducted on these oat-wheat noodles. For Asian noodles, colour is a very important sensory quality. In this study, whole oat flour was used and it had a negative impact on colour of noodles and produced dull noodles with higher specks and lower colour stability in comparison to control noodle samples. In future, further research is needed to understand reasons for variation among oat cultivars in colour stability, and investigate if any residual polyphenol oxidase activity survives in oat groats even after heat processing. Additionally, reasons for non-polyphenol oxidase darkening should also be examined by studying the phenolic compounds or aromatic amino acids which could be responsible for discoloration. To develop a healthy product which is readily accepted by consumers, along with nutritional properties it should also have acceptable sensory characteristics. In future, more research should be conducted to improve the sensory quality of oat-wheat noodles. The bran content in oat flour had a major impact in producing darker noodles with more specks. Selection of oat cultivars with lighter bran could be one way of improving the colour quality and the other way could be by pearling the oat groat samples to remove a small percentage of oat bran, which is darker than the endosperm and devoid of β-glucan. The bran layers however are strongly attached to the aleurone layer of oat grain where the β-glucan content is high. Thus, if higher percentage of bran layer is removed it may cause removal of β-glucan which adheres to the bran layer. Thus, an optimum pearling operation with good balance of improving the sensory properties of products and not removing β-glucan in the process is required and should be an important aim for future research.

The objective of the final study was to determine the physicochemical properties of β-glucan isolated from oat flour and oat-wheat white salted noodles, for the different oat cultivars, and from two growing seasons. In addition, the effect of processing on the susceptibility of β-glucan to breakdown from different oat cultivars and different growing seasons was also evaluated. The β-glucan final viscosity measured, after β-glucan was extracted under physiological conditions (using RVA) from oat flour and 30% OW noodles, varied among different oat cultivars and growing years and was highly correlated to the β-glucan content, extracted β-glucan content and combined effect of β-glucan molecular weight with extract concentration of β-glucan. β-glucan viscosity was highest for Williams and Mitika for oat flour and 30% OW noodle samples for both the years. Bannister 30% OW noodles (2011) and Yallara oat flour and 30% OW noodles for both years were identified as the cultivars with the lowest β-

glucan viscosities. Noodle processing conditions had an impact on β-glucan and reduced its solubility and molecular weight. However, the percentage of breakdown varied among cultivars with Kojonup being least susceptible to breakdown due to processing. This study gives valuable information on how β-glucan viscosity varies among different oat cultivars and how processing conditions can affect β-glucan physicochemical properties, which are related to its viscosity. Therefore, this study highlights the importance of the quality of β-glucan in addition to its content in oat cultivars.

The findings presented in Chapter 6 however are limited and to understand if differences in β-glucan viscosities among oat cultivars, and if the breakdown of β-glucan due to processing has any significant impact on health benefits, in vivo studies are needed. The other limitation of this study was that the molecular weights (M_p and M_w) of β-glucan extracts from the oat flour and 30% OW noodle samples was very high and outside the calibrated molecular weight range. The molecular weights were therefore calculated values which only gave estimated molecular weight (M_p and M_w) values for the sample. In future, β-glucan standards with higher molecular weights need to be produced to generate more accurate results.

Overall, from this research work it can be concluded that although 30% OW noodles with Yallara had good noodle quality in terms of colour and colour stability, they also had the lowest soluble β-glucan viscosity for oat flour and 30% OW noodles. In contrast, Williams produced the least bright noodle, with higher specks and lowest colour stability but had the highest soluble β-glucan viscosity for oat flour and 30% OW noodles for both the years. Furthermore, Mitika 30% OW noodles had the brightest colour, highest colour stability and was superior in other noodle quality attributes in comparison to other oat cultivars studied and also had high soluble β-glucan viscosity. In future, research can be conducted to evaluate how these cultivars selected for this study from different growing seasons perform when they are used for producing different oat food products, such as oat rice, oat beverages, oat cookies, rolled oats and oat breads. Also, resourcing oat cultivars from a wider population such as China and Canada with increased variability in composition, physicochemical properties, β-glucan viscosity and β-glucan molecular weight would help confirm the findings.

Chapter 8: References

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APPENDIX 1

ANNUAL TOTAL RAINFALL FOR THE YEAR 2011 AND 2012 IN KATANNING

1A. Rainfall 2011 (Source: Bureau of Meteorology 2013)

2011	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Graph												
1st	0	0.4	0	0	0	0.6	4.4	1.8	0.2	0	0	0
2nd	0	0	0	0	0	7.4	1.4	4.0	10.2	0.2	0	0
3rd	0	0	0	0	0	0	1.4	3.0	1.4	8.6	0.6	0
4th	0	0	0	0	0	0.2	0.8	2.0	0.2	0.2	45.8	0
5th	0	0	0	0	0	0	0.2	0.2	0.2	0	2.2	1.0
6th	12.4	0	0	0	0.2	0	1.2	0.2	0.2	0	14.0	0
7th	0	0	0	2.2	0	0.2	1.0	1.4	0	0	0	4.0
8th	0	0	0	1.2	0	0.2	0.2	0	0	0	2.2	4.2
9th	0	0	0	0	0	0	0	0.4	0	0.8	4.6	0.8
10th	0	0	0	0	0	0	0	0	0	0	0	0
11th	0	0.2	0	1.0	0	0	1.0	0.2	0.2	0	0	0
12th	0	1.0	0	0.4	0	0	5.8	0	0	0	0	0
13th	0	0	0	0	0	0	0.4	0.2	0	0.8	0	61.8
14th	0	0	0	0	0	6.6	0.2	12.2	0	1.8	0	4.0
15th	0	0	0	0	0	1.8	0	0.2	0	0	2.2	0
16th	0		0	0	0.4	0	10.0	6.8	4.2	0	0	0
17th	0	0	0	0	0.6	0	0	0.2	11.6	0	0	0
18th	0	0	0	1.0	8.0	0	0.2	0.2	12.4	0.2	4.6	0
19th	0	0	1.8	0	0	2.8	0	0	5.6	3.6	0	0
20th	0	0	0	0	3.0	0	0.2	0	0	0	0	0
21st	0	0	0	0	14.6	0	3.2	0.2	1.0	0	0	0
22nd	0	0	0	0	0	0	0.4	21.8	4.8	0	0	0
23rd	5.6	0	2.8	0	0	0	0.6	1.4	0.4	31.0	0	0.2
24th	31.8	0	0	0	0.2	0.2	0.2	1.6	0	0	0	0
25th	8.8	0		12.8	0.2	31.2	0	0.2	0	13.0	0	0
26th	0	0	0	0	0	0.2	3.4	0	3.6	16.6	0	0
27th	0	0	0	5.8	0	0	0.2	0.4	1.2	0	0	0
28th	2.0	0.2	0	2.8	0	4.8	3.2	0.2	4.6	0	0	0
29th	7.2		0	0	0	15.6	7.8	0.2	0	0	0.2	0
30th	30.0		0	0	19.4	0	1.2	0	0	0	0	0
31st	0.2				0.2		12.8	0		0		6.8
Highest Daily	31.8	1.0	2.8	12.8	19.4	31.2	12.8	21.8	12.4	31.0	45.8	61.8
Monthly Total	98.0	1.8	4.6	27.2	46.8	71.8	61.4	58.8	62.0	76.8	76.4	82.8

Annual total for 2011 = 668.4 mm View all monthly data Plot year of daily data

1B. Rainfall 2012 (Source: Bureau of Meteorology 2013)

2012	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Graph												
1st	49.6	0	0	0	0.2	10.0	0	2.8	2.2	0	0	0
2nd	0	0.4	0	0.2	0	1.8	0	0.8	0.4	0	2.2	0
3rd	0	7.6	0	3.0	1.4	0	0	0.2	0.2	0	0	0
4th	0	5.8	0	0	0.6	0	0	0.2	25.0	0	4.4	0
5th	0	0	0	5.4	16.0	0	0	0	1.8	0.2	3.4	0
6th	0	0	0	0.2	10.8	0	0	0	2.2	0	0	34.8
7th	0	0	0	0	0.4	8.2	1.6	11.6	0	0	0.4	0.4
8th	0	0	0	0	9.2	6.4	2.2	0	0.2	0	0	0.4
9th	0	0	0	0	0	0.2	0.2	0	0	0.4	0	0
10th	0	0	0	0	0	0	0.2	0.2	0	0	0	0
11th	0	0.4	0	0	0	18.0	10.0	0	2.0	0	0.2	0
12th	0	0	0	0	0	3.0	0.2	0.8	0.2	0	0	1.2
13th	0	0	0.2	0	0	16.8	0.6	10.2	0	0	0	41.4
14th	0	0	0	0	0.2	0.6	0.8	0	0	2.8	0	0
15th	0	0	0	0	0	0.8	0.2	3.2	0.2	0	0	0
16th	0	0	0	0	0.2	1.0	0.6	0	0	0.4	0	0
17th	0	0	0	0	0	0	0.2	0	0	0	0	0
18th	0	0	0	0	0.2	0	0	0.2	1.8	0	0	0
19th	0	0	0	2.2	0	8.4	0	0	0.2	0	0	0
20th	16.8	0	0	0	0	3.2	0	0.6	0.2	0	0	0
21st	0	0	0	0	0.2	2.2	0.2	1.2	0.2	0	0	0
22nd	0.6	0	0	0.2	0	0	0	9.6	6.0	4.6	0	0
23rd	0	0	0	0	0	0	0.2	0	0.2	2.4	0	0
24th	0	0.2	0	0	0	0	3.2	1.8	0	0	0	0
25th	0	0	3.4	0	0	0	0	0.2	1.6	0	2.0	0
26th	0	0	0	0	0	0	0.4	0	2.0	0	0	0
27th	0	0	0	0	0	2.4	0	3.2	19.2	0	0	0
28th	0	0	0	0.2	0	0	0	0.2	5.6	0	0.8	0
29th	0	0	0	0	0	0.2	0.4	1.0	0	0	11.4	0
30th	0		0	2.0	0	0	0	0.2	0.2	0	3.6	0
31st	0		0		0		1.6	0		0		0
Highest Daily	49.6	7.6	3.4	5.4	16.0	18.0	10.0	11.6	25.0	4.6	11.4	41.4
Monthly Total	67.0	14.4	3.6	13.4	39.4	83.2	22.8	48.2	71.6	10.8	28.4	78.2

Annual total for 2012 = 481.0 mm View all monthly data Plot year of daily data

APPENDIX 2

ANNUAL MEAN MAXIMUM TEMPERATURE FOR THE YEAR 2011 AND 2012 IN KATANNING

2A. Temperature 2011 (Source: Bureau of Meteorology 2013)

2011	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Graph												
1st	29.6	25.4	27.1	27.3	21.3	18.9	14.3	17.0	19.0	24.1	26.2	28.4
2nd	33.3	28.6	28.2	26.5	20.8	15.2	10.8	17.1	15.0	20.4	31.0	29.2
3rd	36.1	32.8	29.7	27.7	21.9	14.6	11.4	11.4	15.1	17.8	30.1	31.6
4th	37.2	28.3	34.5	30.4	23.9	14.9	12.3	13.2	17.5	19.3	23.1	34.2
5th	36.5	21.4	29.5	32.8	23.6	15.3	11.7	12.1	16.0	20.0	20.3	30.5
6th	28.1	23.5	26.8	33.2	18.6	15.9	13.1	14.5	16.6	22.1	21.0	22.8
7th	26.4	28.5	25.9	20.1	20.3	16.5	12.8	17.1	14.7	22.6	22.1	21.4
8th	29.4	26.5	27.4	19.6	25.2	16.9	12.5	16.5	15.6	20.7	15.9	21.3
9th	33.9	30.7	30.7	19.7	20.4	17.0	12.8	14.5	15.8	19.2	20.7	22.3
10th	30.6	22.9	36.0	22.9	19.7	16.9	12.4	15.0	17.3	22.0	23.3	26.3
11th	30.1	24.3	27.4	23.9	19.5	16.7	9.4	15.6	20.8	26.7	24.7	30.9
12th	32.9	22.4	24.4	21.8	19.0	17.2	11.5	17.1	23.7	20.1	24.2	22.6
13th	39.8	27.7	26.0	23.2	19.2	15.4	15.7	19.6	16.5	19.5	24.4	19.4
14th	26.7	27.6	29.4	22.2	20.1	11.8	17.3	14.8	19.4	22.4	23.4	22.2
15th	28.8		27.9	26.5	19.6	16.1	14.8	16.0	19.7	24.0	22.4	21.7
16th	31.8	27.2	24.7	31.1	21.1	16.6	14.3	12.6	19.9	32.5	27.8	25.6
17th	38.7	26.3	24.9	24.2	21.7	16.8	14.1	15.3	16.4	23.6	25.5	27.7
18th	30.1	29.1	30.0	21.7	17.9	17.4	15.0	14.5	13.1	19.9	21.6	28.4
19th	34.3	26.2	27.1	21.2	20.4	17.0	12.8	18.1	16.8	18.8	22.0	25.1
20th	26.3	23.4	24.4	22.4	15.3	17.5	16.3	20.1	19.7	22.0	25.6	28.7
21st	33.3	31.9	26.4	23.1	14.9	14.7	14.2	23.5	14.7	27.7	25.7	35.4
22nd	40.1	33.0	31.7	28.5	16.3	17.7	15.9	15.2	13.7	28.5	28.1	33.9
23rd	20.4	31.4	30.1	30.1	18.0	18.1	15.5	16.2	17.0	19.7	33.5	27.5
24th	17.3	32.8	29.2	25.3	16.3	19.8	17.9	14.7	19.8	15.4	34.8	26.9
25th	24.4	36.2	30.7	22.8	16.4	15.0	16.6	13.9	19.7	14.6	33.8	26.5
26th	30.8	33.4	32.2	25.5	14.5	16.8	16.9	15.2	14.4	21.9	18.1	27.9
27th	34.8	34.5	32.3	20.4	17.1	18.4	17.4	16.7	12.9	23.1	23.6	31.6
28th	43.5	25.9	30.4	17.4	21.9	12.9	17.2	18.1	16.6	18.8	22.9	33.8
29th	32.8		26.5	19.2	24.7	14.6	16.1	18.5	17.9	20.9	20.1	37.6
30th	26.2		26.8	20.6	18.8	14.3	17.3	16.1	19.6	22.4	24.4	35.3
31st	20.8		27.6		18.4		15.7	18.8		24.9		31.2
Highest daily	43.5	36.2	36.0	33.2	25.2	19.8	17.9	23.5	23.7	32.5	34.8	37.6
Lowest daily	17.3	21.4	24.4	17.4	14.5	11.8	9.4	11.4	12.9	14.6	15.9	19.4
Monthly mean	31.1	28.2	28.6	24.4	19.6	16.2	14.4	16.1	17.2	21.8	24.7	28.0

Annual mean maximum temperature for 2011 = 22.5 °C View all monthly data Plot year of daily data

2B. Tempertature 2012 (Source: Bureau of Meteorology 2013)

2012	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Graph												
1st	21.8	37.4	25.6	23.7	19.2	14.7	16.9	14.2	17.9	29.5	14.9	23.3
2nd	25.1	26.7	25.6	22.7	22.5	20.1	18.4	14.9	18.2	27.8	24.8	25.7
3rd	24.0	17.6	28.0	26.5	26.9	21.3	17.8	14.4	17.8	18.9	24.5	28.6
4th	23.9	19.9	32.7	26.1	17.3	19.9	17.5	16.8	10.7	19.1	19.1	33.7
5th	32.7	21.4	36.5	17.6	20.4	18.8	19.1	16.0	16.4	18.8	19.4	34.7
6th	26.4	24.8	35.5	19.4	16.3	17.8	14.6	17.4	14.3	25.4	23.7	28.6
7th	23.1	28.0	31.7	23.9	18.2	15.4	14.0	13.8	15.7	23.7	23.7	23.0
8th	23.1	28.7	27.6	29.1	16.4	18.1	11.6	14.3	19.7	21.4	24.4	24.9
9th	26.3	30.3	32.0	31.7	18.9	18.5	12.9	15.9	24.3	21.1	32.1	31.7
10th	27.9	30.8	38.8	32.5	18.9	17.2	11.2	19.7	22.2	23.9	27.6	35.5
11th	30.9	34.3	36.7	32.5	18.3	15.3	12.6	22.3	18.2	27.4	21.4	31.6
12th	32.5	26.5	39.1	28.8	22.1	14.2	13.1	17.0	15.5	30.1	26.1	19.7
13th	32.8	29.2	24.1	25.9	22.7	10.6	13.5	12.4	17.6	25.6	30.1	20.2
14th	30.8	32.8	22.5	23.4	19.1	13.3	15.5	15.7	20.3	21.0	26.9	24.2
15th	34.5	35.7	24.3	25.9	19.5	14.6	17.3	13.7	23.1	21.8	28.4	27.9
16th	25.3	34.1	26.1	25.9	18.8	15.9	15.1	14.0	22.3	23.2	29.6	30.3
17th	27.0	31.7	30.8	25.2	19.3	17.5	12.1	15.1	26.3	25.2	34.5	32.6
18th	32.8	27.8	31.7	24.1	18.5	18.7	12.8	18.4	20.5	25.2	32.6	27.8
19th	36.5	27.9	25.4	18.1	21.4	16.0	15.6	19.4	19.1	24.3	28.1	27.1
20th	32.5	31.0	21.0	18.1	22.4	12.0	17.0	13.7	20.6	24.9	28.2	32.5
21st	37.8	35.5	21.7	19.1	20.4	11.9	19.1	17.2	18.9	33.4	33.0	37.9
22nd	35.7	37.7	22.3	17.8	18.6	14.2	17.1	12.6	15.2	21.3	35.4	28.0
23rd	29.7	37.0	23.9	16.3	16.3	14.7	17.0	16.2	16.0	20.5	26.1	29.1
24th	29.4	26.5	25.6	21.2	17.7	14.6	11.5	15.4	21.7	20.4	26.0	30.4
25th	35.0	26.9	25.4	24.9	17.9	13.3	13.2	18.3	18.9	21.2	26.1	37.3
26th	37.4	22.3	27.6	27.7	20.7	15.8	13.5	18.3	15.0	24.9	30.3	32.9
27th	40.1	26.8	26.4	23.6	20.9	16.1	15.0	14.9	12.2	29.7	35.5	34.2
28th	39.9	22.7	25.4	22.9	19.1	14.4	15.8	17.3	13.6	35.6	21.3	37.8
29th	24.7	25.1	31.4	22.8	20.0	14.2	16.3	16.1	17.3	27.6	15.6	38.9
30th	24.0		21.6	19.8	22.4	16.3	18.3	19.1	23.9	21.1	20.0	40.5
31st	29.0		23.1		19.1		17.0	21.7		18.3		40.0
Highest daily	40.1	37.7	39.1	32.5	26.9	21.3	19.1	22.3	26.3	35.6	35.5	40.5
Lowest daily	21.8	17.6	21.0	16.3	16.3	10.6	11.2	12.4	10.7	18.3	14.9	19.7
Monthly mean	30.1	28.9	28.1	23.9	19.7	15.8	15.2	16.3	18.4	24.3	26.3	30.7

Annual mean maximum temperature for 2012 = 23.1 °C View all monthly data Plot year of daily data

APPENDIX 3

OFFICIAL JAPANESE METHOD FOR PROCESSING WHITE SALTED UDON NOODLES (Source: AEGIC)

Formulation based on

400g flour (adjusted for moisture content)

8 g of NaCL

34% of water (adjusted for flour moisture content)

Weigh the appropriate amount of flour for the first sample (the warm-up).

Weigh the required water at 27^{o}C into the 500mL flask and dissolve one of the pre-weighed 8g of NaCl. This should give dough crumb at 25 to 26°C when room temp is 23°C.

Remove the bowl from the mixer and transfer the flour into it. Refit the bowl and switch on using speed 1.

Add the salt solution by letting it trickle down the side of the bowl over a period of 30sec. Allow mixing to proceed for one minute.

At the end of the minute stop the mixer change to speed 2, lower the bowl and remove any dough adhering to the bottom of the bowl or the beater.

Raise the bowl, switch on and re-start the clock for a further minute (1min).

Stop mixer, change back to speed 1 and mix for three more minutes (3min). Monitor the mixing during this time to ensure the crumb does not form a lump around the beater.

After 3 minutes switch the mixer off.

Remove the bowl, insert the digital thermometer into the dough and record the temperature. It should be 26°C. Examine the crumb make notes on the work sheet regarding its lumpiness and moisture status.

PREPARATION OF THE DOUGH SHEET

Safety Precaution – Disconnect the clutch lever which activates the cutting rolls. This lever may accidentally be pushed in during sheeting, causing the rolls to operate unbeknown to the operator.

While the warm-up sample is being prepared turn the noodle machine on and carefully wipe the rollers from underneath with a warm dishcloth. Dry them with a tea towel. Clean and dry the dough chute also.

Put on disposable gloves.

The warm-up sample is used to 'condition' the noodle machine and pick up any dirt or grease that may be on the rollers or the cutters. Put the dough sheet through the cutters several times by re-forming the dough sheet. Discard the warm-up sample and set the roll gap at 3mm. When setting the rolls always exceed the gap width required and then close down the adjusting wheels to reach the measurement needed. DO NOT change settings while the rolls are in motion.

With the catching tray in position, place two compacted handfuls of crumb from the first sample in the roll gap.

Empty the crumb of the first sample from the mixing bowl onto the rolls. Spread it evenly over the roll gap. Lower the Perspex guard and press the green ON switch. As the crumb passes through, sweep any loose tailings down the dough chute.

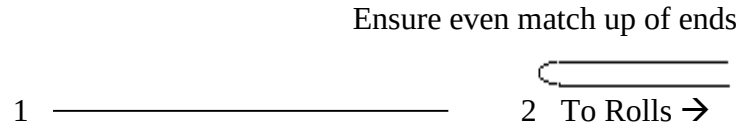
Take hold of the emerging sheet and prepare to catch the end of it and gather any tailings in the middle of the sheet. Lay the sheet in the chute.

Empty the material in the catching tray into the middle of the sheet.

Fold the sheet as illustrated:

Pass through the rolls a second time and collect the sheet with both hands.

Fold in half matching up the two ends as closely and evenly as possible and feed to the rolls as illustrated.



Feed the emerging sheet onto a plastic roller from the Contherm cabinet, place in a large plastic bag and expel the air as it is rolled up.

Label with the sample number and the current time. Set the roll aside in the Contherm cabinet to rest for 1 hour (set the digital timer).

Proceed mixing and sheeting the remaining test samples.

AFTER RESTING

When one hour has elapsed retrieve the first sample from the Contherm cabinet and remove the plastic bag.

Reduce the roll gap to 2.5mm, pass the sheet through and collect it on the roll.

Reduce the roll gap to 2.2mm, pass the sheet through again, collect it on the roll and transfer it to the clean bench top.

Lay the sheet out and measure the length down each side and along the centre.

Record the average length on the work sheet.

Measure the sheet thickness along each side using Peacock gauge No. 2. Fold the sheet gently lengthwise to assist with this task.

Record the average thickness on the work sheet.

PRODUCING SHEETS FOR COLOUR TEST AND JUDGING

Reduce the roll gap to 1.9mm.

Cut a 7cm long piece off the end of the sheet with the large knife.

Put it through the rolls and catch it as it emerges.

Cut off both ends and both edges with the scissors to end up with a centre portion of the sheet approximately 3cm X 7cm.

Place this between the watch glasses used to calibrate digital Peacock gauge No. 1 and measure the thickness. It must be 2.5mm ± 0.05mm thick. If it is not, adjust the roll gap and trial another piece until the sheet is at the desired thickness.

When satisfied, cut a piece 14cm long and pass it through the rolls. Lay it on the bench top and cut it exactly in two. Place one half on top of the other and hand to operator 1 for the Minolta colour reading.

Cut a 2nd sheet 11cm long. Pass it through the rolls, place in the first plastic bag prepared for judging sheets, fold the top over and place in the fridge.

CUTTING STRANDS

Switch on the noodle machine and engage the cutters by pushing in the clutch lever. Move the guiding plate to the cutter position.

Roll the remaining length of sheet onto the roller and position it in the central holders above the dough chute. Unwind the sheet and guide the end into the rolls. Close the guard and switch on, carefully assist the sheet into the rolls if necessary. Beware the end of the sheet folding over as it comes off the roller.

Quickly move to the front of the machine and, rejecting the two outside strands on each side, take hold of the strands as they emerge.

Transfer them to the cutting board, dust liberally with starch particularly at the 25cm marks and cut into lengths.

Check there are no strands sticking together, fold and place in the first bag prepared for raw noodles. Proceed with the other samples in turn until all have been completed. Set them aside on the window ledge to await cooking.

APPENDIX 4

WATER TABLE FOR JAPANESE NOODLE PROCESSING

(Source: AEGIC)

JAPANESE NOODLES			
FLOUR and WATER Wts.			
for 400g Mix at 13.5% m.c.			
			8.0 g NaCl
Flour	Flour Wt.	Water Wt.	
m.c. %	g	g	
10.6	387	149	
10.7	387	149	
10.8	388	148	
10.9	388	148	
11.0	389	147	
11.1	389	147	
11.2	390	146	
11.3	390	146	
11.4	391	145	
11.5	391	145	
11.6	391	145	
11.7	392	144	
11.8	392	144	
11.9	393	143	
12.0	393	143	
12.1	394	142	
12.2	394	142	
12.3	395	141	
12.4	395	141	
12.5	395	141	
12.6	396	140	
12.7	396	140	
12.8	397	139	
12.9	397	139	
13.0	398	138	
13.1	398	138	
13.2	399	137	
13.3	399	137	
13.4	400	136	
13.5	400	136	
13.6	400	136	
13.7	401	135	
13.8	401	135	
13.9	402	134	
14.0	402	134	
14.1	403	133	
14.2	403	133	
14.3	404	132	
14.4	404	132	
14.5	405	131	
Flour Wt. = (100-13.5)/(100-m.c.)*400			
Water Wt. = 136+(400-Flour Wt.)			

APPENDIX 5

ADDITIONAL TABLE OF CHAPTER FOUR

Variation in Lipid and Amylose Content of Oat Flour Cultivars

Cultivars	Lipids[#*] %	Amylose[#*] %
Mitika	7.3c ± 0.45	19.8d ± 0.26
Kojonup	6.2d ± 0.53	21.5b ± 0.60
Yallara	5.6e ± 0.13	21.0bc ± 0.70
Bannister	8.9a ± 0.27	22.4a ± 0.38
Carrolup	6.4d ± 0.41	20.6cd ± 0.14
Williams	8.3b ± 0.36	21.6b ± 0.10

[a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$);
[#] Average of a cultivar over 2 years

APPENDIX 6

ADDITIONAL TABLES OF CHAPTER FIVE

6A. RVA Pasting Properties of 30% Oat-Wheat Blends

Cultivars	Setback [#] (cP)	Peak Time [#] (Min.)	Pasting Temperature [#] (° C)
Mitika	1429ab ± 37.0	6.16a ± 0.05	69.7ab ± 0.23
Kojonup	1418bc ± 13.3	6.05b ± 0.06	70.0a ± 0.41
Yallara	1401c ± 20.5	6.04b ± 0.03	69.6abc ± 0.23
Bannister	1378d ± 13.5	6.02bc ± 0.05	69.5bc ± 0.05
Carrolup	1401c ± 18.4	6.04b ± 0.02	69.4bc ± 0.18
Williams	1447a ± 9.4	5.98c ± 0.02	69.3c ± 0.40

[a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); [#] Average of a cultivar over 2 years

6B.CIE (Δ *L**) Value of Noodle Sheet (0-24 hr)

Cultivars	2011#	2012#
Mitika	10.77 ± 0.16	10.18 ± 0.67
Kojonup	11.36 ± 0.46	10.60 ± 0.42
Yallara	11.10 ± 0.12	9.96 ± 0.41
Bannister	11.96 ± 0.11	11.01 ± 0.22
Carrolup	11.58 ± 0.37	11.36 ± 0.14
Williams	11.89 ± 0.13	11.60 ± 0.12

[a] Mean values ± (± standard deviation); # Year and cultivar interaction was not significant (P>0.05)

6C. Firmness value of 30% OW cooked noodles

Cultivars	2011#	2012#
Mitika	875 ± 14.1	992 ± 30.4
Kojonup	896 ± 21.2	988 ± 8.5
Yallara	852 ± 17.0	1012 ± 19.1
Bannister	871 ± 7.8	943 ± 29.3
Carrolup	885 ± 35.4	989 ± 68.6
Williams	880 ± 28.3	1009 ± 3.5

[a] Mean values (± standard deviation); # Year and cultivar interaction was not significant (P>0.05)

6D. Cooking Loss (%) (Solid Loss in Cooking Water) of 30% OW noodles

Cultivars	2011#	2012#
Mitika	4.65 ± 0.07	4.29 ± 0.01
Kojonup	4.67 ± 0.02	4.45 ± 0.07
Yallara	4.48 ± 0.18	4.46 ± 0.09
Bannister	4.70 ± 0.00	4.45 ± 0.21
Carrolup	4.50 ± 0.07	4.40 ± 0.00
Williams	4.80 ± 0.07	4.60 ± 0.07

[a] Mean values (± standard deviation); # Year and cultivar interaction was not significant (P>0.05)

APPENDIX 7

ADDITIONAL TABLES OF CHAPTER SIX

7A. Supernatant Viscosity of oat flour sample using viscometer

Cultivars	2011#	2012#
Mitika	275a ± 21.2	288b ± 10.6
Kojonup	145c ± 7.1	260c ± 0 *
Yallara	105d ± 0.	140e ± 0*
Bannister	148c ± 10.6	220d ± 7.1*
Carrolup	195b ± 7.1	200d ± 14.1
Williams	265a ± 21.2	393a ± 3.5*

[a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); # Year and cultivar interaction was significant (P>0.05)

7B. Supernatant Viscosity of 30%OW noodle sample using viscometer

Cultivars	2011#	2012#
Mitika	16.30a ± 0.42	17.35b ± 0.21*
Kojonup	12.10b ± 0.14	16.75b ± 0.35*
Yallara	11.15c ± 0.21	12.25c ± 0.35*
Bannister	10.65c ± 0.21	14.15d ± 0.21*
Carrolup	12.30b ± 0.71	12.25c ± 0.35
Williams	16.50a ± 0.14	30.25a ± 0.35*

[a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); # Year and cultivar interaction was significant (P>0.05)

7C. β-glucan content of 30% OW noodle extract

Cultivars	β glucan % of extract (Noodles)#*
Mitika	1.21b ± 0.03
Kojonup	1.07cd ± 0.11
Yallara	0.99e ± 0.09
Bannister	1.06d ± 0.07
Carrolup	1.09c ± 0.02
Williams	1.30a ± 0.08

[a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); # Year and cultivar interaction was not significant (P>0.05); *The cultivar differences were significant

7D. Peak and average molecular weight of 100% oat flour

Cultivars	M_p of extracted β-glucan (Flour)#*	M_a of extracted β-glucan (Flour)#*
Mitika	2781a ±48.85	1939a ±50.82
Kojonup	2755ab ±60.34	1941a ±60.27
Yallara	2693c ±15.87	1859b ±16.96
Bannister	2705bc ±76.11	1891b ±60.29
Carrolup	2753ab ±43.71	1972a ±40.04
Williams	2752ab ±65.81	1950a ±45.99

[a] Mean values (± standard deviation) in the same column with different letters are significantly different (P<0.05); # Year and cultivar interaction was not significant (P>0.05); *The cultivar differences were significant

7E. Percent decrease in peak molecular weight after processing

Cultivars	2011#	2012#
Mitika	59.8bc ± 0.56	62.5a ± 0.21*
Kojonup	56.9d ± 0.56	58.4b ± 0.14*
Yallara	58.9c ± 0.45	62.3a ± 0.07*
Bannister	61.6a ± 0.61	62.8a ± 0.78*
Carrolup	61.3a ± 0.88	62.4a ± 0.42*
Williams	60.9ab ± 0.55	58.9b ± 0.9*

[a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$); # Year and cultivar interaction was significant ($P>0.05$)

7F. Percent decrease in average molecular weight after processing

Cultivars	2011#	2012#
Mitika	36.9c ± 0.40	42.4b ± 0.11*
Kojonup	32.7d ± 0.54	36.4c ± 0.22*
Yallara	35.9c ± 0.43	43.4ab ± 0.26*
Bannister	44.2a ± 0.92	44.8a ± 0.68
Carrolup	44.1a ± 1.22	43.2ab ± 0.71
Williams	41.4b ± 0.01	35.2c ± 1.70*

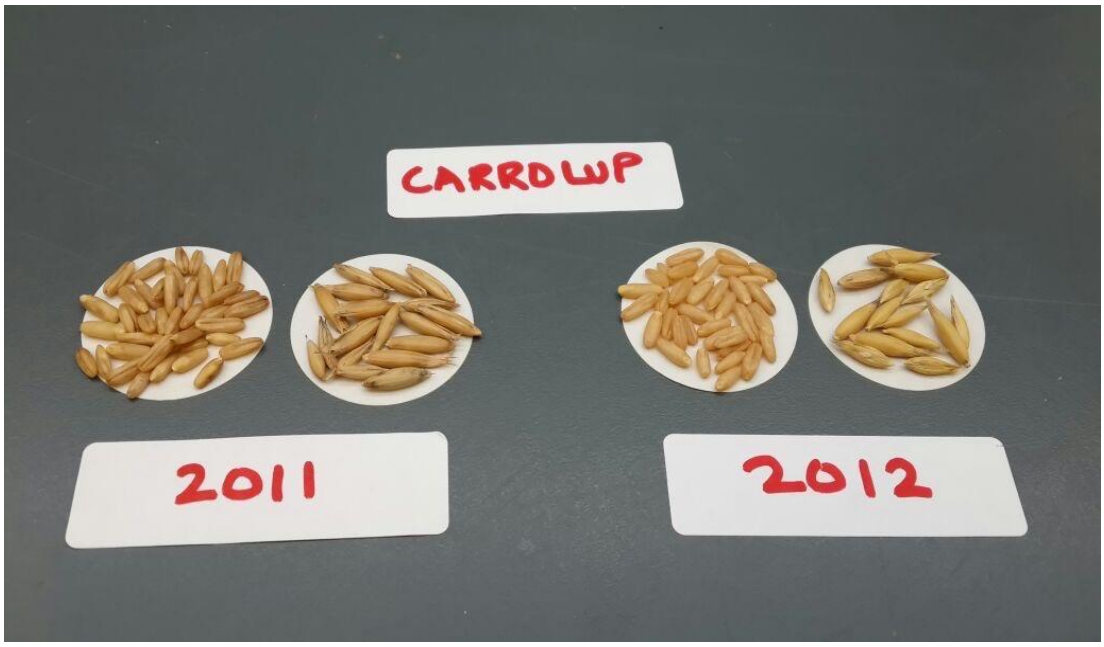
[a] Mean values (± standard deviation) in the same column with different letters are significantly different ($P<0.05$); # Year and cultivar interaction was significant ($P>0.05$)

APPENDIX 8

PHOTOGRAPHS OF OAT GRAINS AND GROATS


YALLARA
2011
2012


BANNISTER
2011
2012


CARRDLUP
2011
2012


WILLIAMS
2011
2012

APPENDIX 9

PHOTOGRAPHS OF COLOR OF RAW NOODLE SHEET AT 0 H

APPENDIX 10

PHOTOGRAPHS OF COOKED NOODLES (15 MIN AFTER BOILING)