School of Public Health

Defining the wheat quality requirements for Indian whole wheat chapatti

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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.

Signature: ………………………………………….

Date: ……………………….............................

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ABSTRACT

Australia is a major wheat exporting country and exports approximately two thirds of its annual wheat production, worth over five billion Australian dollars. India has been identified as an important growth market for Australian wheat exports. India is currently the second largest producer and consumer of wheat in the world after China. Ninety percent of the wheat produced in India is used to make their staple food chapatti. India's wheat production however, has begun to plateau and the issue of food security and the ability to feed an increasing population has been the renewed topic of discussion. Grain which can meet customer requirements by producing quality end products will be essential in developing an export market of Australian wheat to India. Thus the research investigated the ability of Australian wheat to make the staple Indian food chapatti; and also provided further understanding of wheat quality requirements for chapatti.

The first stage of the research identified and selected suitable Australian wheat varieties for chapatti making and quality evaluation. Australian wheat varieties were harvested from five National Variety trials in W.A. and two trials, Binnu and Williams, were selected for study. From the two trials, twenty commercially released Australian hard wheat varieties and five commercially released Australian soft noodle wheat varieties were evaluated. Six Indian wheat varieties were also studied and used as benchmarks. Two of the Indian wheat varieties, *HI 1531* and *PBW 175,* were used as gold standards. The grain samples were stone milled producing chapatti flours of high extraction, of one hundred percent minus coarse bran particles.

The second stage characterised the physicochemical and rheological properties of the Australian and the Indian chapatti flours using standard wheat quality tests. In addition, the wheat proteins; high molecular weight glutenin subunits, low molecular weight glutenin subunits, albumins and gliadins, were profiled using matrix assisted laser desorption/ ionisation time of flight mass spectrometry to further characterise chapatti flour quality. The third stage involved making the Australian and the Indian chapatti flours into chapattis using a standard chapatti making method; and chapatti quality was evaluated by measuring chapatti quality characteristics including puffing ability, chapatti colour and textural attributes. Lastly, selected Australian and the

Indian chapatti flours were made into chapatti using a different chapatti making method; and the chapatti quality was evaluated to investigate the importance of the chapatti making method on quality and further understand relationships between flour quality and chapatti quality.

Flour quality and chapatti quality was significantly correlated but the Australian hard wheat varieties from the Binnu and the Williams trials were not closely related to the Indian wheat varieties. The measurement of flour protein content, damaged starch content, water absorption, flour colour and dough extension were predictors of chapatti quality. Flour quality requirements of high damaged starch and high water absorption were found to be important; and confirmed the previously reported wheat quality requirements for chapatti. Higher damaged starch is important for facilitating greater water absorption of the flour to achieve dough of suitable consistency for kneading and sheeting; and water for steam leavening of the chapatti.

The flour and chapatti quality comparisons with the Indian wheat varieties showed that hard wheat types are needed for chapatti making, and not soft wheat types. The requirement of medium hard grain for chapatti making was demonstrated in this research, by the finer particle size of the Indian wheat varieties in comparison to the Australian hard wheat varieties. The grain hardness of the Indian hard wheat varieties appears to be unique, and not as 'hard', as the Australian hard wheat varieties studied.

Protein content of ten to twelve percent and low dough extension properties were also identified as being important for chapatti making. Dough which is easy to knead and sheet is desirable for chapatti making; therefore dough should not be overly strong or elastic. The Indian wheat varieties were observed to have these dough handling attributes but the Australian hard wheat varieties had greater recoil and elastic properties when sheeting. In addition, HMW-GS 2+12 located at the *Glu-D1* loci were determined to be present in the Indian wheat varieties known to make good quality chapatti and therefore may have potential as a preliminary screening quality trait. Specific combinations of HMW-GS however are thought to have more importance for chapatti quality and in relation to other proteins. The Australian wheat varieties shown to make acceptable quality chapatti had some similarities in

HMW-GS and gliadin protein composition to the Indian wheat varieties which make good quality chapatti.

Two chapatti making methods were trialled, based on Indian chapatti making methods, and the Australian wheat varieties generally made better quality chapattis with the first laboratory chapatti making method. Australian hard wheat varieties *Bumper*, *Correll*, *Espada*, *Fang*, *Gladius* and *Magenta* were found to have similar textural properties, having softer texture, as Indian wheat varieties *HI 1531* and *PBW 175*; from the first method. In addition, *Bumper* had similar chapatti colour to the Indian wheat varieties, in particular *PBW 175*, and the lowest extensibility of the Australian wheat varieties studied. The objective chapatti quality traits measured, including puffing characteristics, chapatti colour and textural attributes, were all shown to be important for describing chapatti quality.

Furthermore, the use of sensory assessment, conducted in the second chapatti making study, was shown to be valuable for differentiating the chapatti quality of wheat varieties. The Australian hard wheat varieties *Bumper*, *Mace*, *Magenta* and *Tammarin Rock* had the highest sensory assessment scores, of the Australian wheat varieties, when made using the second chapatti making method. The development and use of two different chapatti making methods highlighted the effect the chapatti making method has on different chapatti quality traits.

Overall the Australian wheat varieties were not able to make chapatti of the same quality as the Indian wheat varieties, which made good quality chapatti. The ability of the Australian wheat varieties however to make acceptable quality chapattis, despite significant differences in flour quality, to the Indian wheat varieties, means they are likely to have the potential to make better quality chapatti with careful selection of flour quality attributes and chapatti making methodology.

In this research, study of the chapatti making abilities of a range of Australian wheat varieties, which had varying wheat qualities, provided insight into the important flour quality, chapatti quality and chapatti making methodology needed for future selection of wheat for the Indian market.

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2. **2010 - International conference presentation (included some results from my PhD)**

   AACC International Annual Meeting, October 24 – 27, 2010, Savannah International Trade and Convention Centre, Savannah, Georgia, U.S.A.

   Fenton, H., Solah, V. A., Johnson, S.K., Cato, L., Williams, H. G. and Crosbie, G.B.

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3. **2010 - International conference poster presentation**

   AACC International Annual Meeting, October 24 – 27, 2010, Savannah International Trade and Convention Centre, Savannah, Georgia, U.S.A.

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   Fenton, H., Solah, V. A., Ma, W. and Williams, H.G.

   'Characterisation of HMW-GS and LMW-GS in Western Australian and Indian wheat cultivars and their relationship to chapatti quality.'

5. **2010 - National conference poster presentation and published conference proceedings paper**

   Fenton, H., Solah, V. A., Williams, H. G., Gujral, H. S. and Crosbie, G. B. Characterisation of good quality chapatti flour. Cereals 2010. Proceedings of the 60th Australian Cereal Chemistry Conference, 20th – 22nd September 2010, Melbourne, Victoria. *In press.*

6. 2009 - National conference poster presentation and published conference proceedings paper

Fenton, H., Ma, W., Solah, V. A. and Gujral, H. S. Profiling HMW-GS in wheat varieties with different chapatti quality. Cereals 2009. Proceedings of the 59th Cereal Chemistry Conference, 27th – 30th September 2009, Wagga Wagga, New South Wales.

7. 2008 - National conference oral presentation and published conference proceedings paper

Fenton, H., Solah, V., Williams, H., Crosbie, G. B., Diepeveen, D., Gujral, H. S. and Ma, W. Characterisation of an Indian whole wheat flour suitable for making good quality chapati. Cereals 2008. Proceedings of the 58th Cereal Chemistry Conference, 31st August - 4th September 2008, Surfer's Paradise, Queensland.

1.0 INTRODUCTION

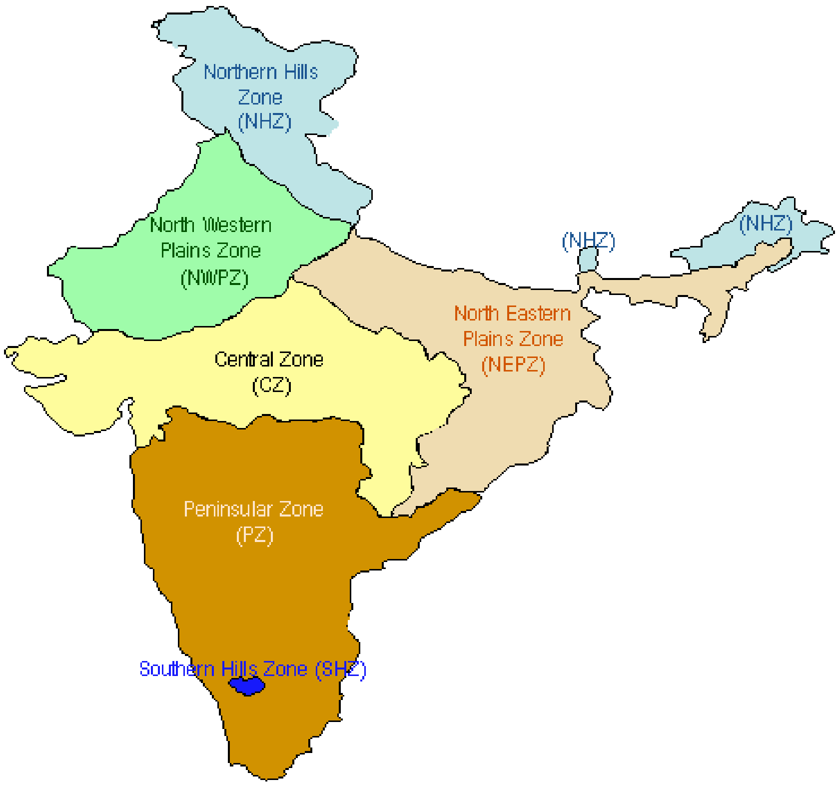
1.1 Wheat production in India

India is currently the second largest wheat producing country in the world; yielding 80.71 million tonnes in 2010 (FAOSTAT 2012). In addition, India is the second largest consumer of wheat in the world after China; with an annual per capita consumption of 60.2 kg in 2007 (FAOSTAT 2010b; Joshi et al. 2007). Of the wheat produced in India, 90% is used to make the staple food chapatti (Yadav et al. 2008a; Ghodke and Ananthanarayan 2007). Since 2000, India's wheat production has begun to stabilise, which is of concern as short term solutions to increase yield are unlikely to be sufficient to sustain the requirements of an increasing population.

In India, wheat is grown as a winter cereal during the cooler, drier, non-monsoon months spanning November to mid-April (Nagarajan 2006). *Triticum aestivum* L. is the main wheat species grown, on approximately 25 million hectares (Nagarajan 2006). *Triticum dicoccum* and *Triticum turgidum* subsp. *durum* are also planted, with the total growing area approximately 27.75 million hectares (FAOSTAT 2010a). Differences in the growing environment across the country has led to the definition of six wheat growing zones (Joshi et al. 2007). The North Western Plain Zone (NWPZ) is the most productive of the agro-climatic zones. It accounts for 80% of wheat produced in India, with the states of western Utter Pradesh, Haryana and Punjab the major contributors (Nagarajan 2004b). The remaining wheat growing zones are the Northern Hill Zone (NHZ), the North Eastern Plain Zone (NEPZ), the Central Zone (CZ), the Peninsular Zone (PZ) and the Southern Hill Zone (SHZ); shown in Figure 1.1.

Currently, India yields approximately 11% of the world's wheat production and this present status has been credited to the Green Revolution. Since 1965, a major turning point in India's agricultural history, the Green Revolution facilitated the change to self-sufficiency in food grain production (MacAulay 2010; Nagarajan 2004b). A difference of 65.5 million tonnes of wheat was produced in 2005 as compared to 1950 (Joshi et al. 2007). One of the most important changes was the introduction of high yielding, semi-dwarf wheat varieties, as opposed to the tall straw land race cultivars that were traditionally grown and are unsuitable for intensive farming (Joshi et al. 2007; Nagarajan 2004b). Currently, India's wheat production has begun to plateau, and the issue of food security and the ability to feed an increasing population has been the renewed topic of discussion (Chatrath et al. 2007; Joshi et al. 2007; Nagarajan 2005). Factors identified as affecting yield include; a decrease in the application of suitable fertilisers, a decrease in the profit to farmers, a need to improve infrastructure, and a need to improve the wheat genetic material available (Nagarajan 2005). Furthermore, environmental issues such as terminal heat stress and the occurrence of new crop diseases, are challenging the ability of India to sustain continued growth in wheat production (Joshi et al. 2007).

Figure 1.1. The six wheat growing zones in India.



Source: (KrishiSewa)

In the development of new Indian wheat varieties for farmers, the aims continue to be for high yield and disease resistance. Consequently, it should be noted that the current wheat varieties grown in India are not the direct result of a coordinated quality breeding program (Joshi et al. 2007). Nonetheless, in recent years with economic growth, urbanisation and the emergence of a middle class population, quality has and will become more important to Indian consumers (MacAulay 2010; Joshi et al. 2007). Over the last two decades, food and cereal research in India has been more prevalent, with greater focus on better understanding relationships between Indian wheat quality and end products (Hemalatha et al. 2007; Sharma et al. 2004; Prabhasankar and Manohar 2002). Furthermore, grade and class systems segregating wheat based on quality have been recently developed (Chatrath et al. 2007; Nagarajan 2006). Breeding for specific quality traits and end-use has been recognised as a potential, positive direction for Indian farmers to receive better prices for wheat grown (Joshi et al. 2007).

Chapatti is a staple food in the diet of the Indian population and is flat unleavened bread made from whole wheat flour and water. Chapattis are traditionally prepared fresh at mealtimes as they stale rapidly; and they are preferred to be consumed while still warm (Haridas Rao, Leelavathi, and Shurpalekar 1986b; Venkateswara Rao et al. 1986). Chapatti are usually torn into smaller pieces and made into a scoop to eat with meat and vegetable curries (Shaikh, Ghodke, and Ananthanarayan 2007). Whole wheat flour, also termed *atta*, is used to make chapatti and the quality of the wheat flour is critical for determining end product quality. Wheat quality requirements for chapatti include a protein content of 10 to 12%, medium dough strength, and properties such as high damaged starch and high water absorption contribute towards good chapatti making (Nagarajan 2006). In India, Indian medium hard bread wheat is the class of wheat most suited to the production of chapatti (Nagarajan 2006).

Whole wheat flour typically contains 95 to 97% of the wheat grain and is a main source of energy, nutrients and fibre for many Indian people (Yadav et al. 2008b). India has a population of 1.2 billion people of which 22% are below the poverty line (FAO 2010; FAOSTAT 2009). Accordingly, the need to provide an economic and nutritious food, like chapatti, is of importance. It has been estimated that India's wheat production needs to increase by 2 to 2.5% a year to cope with the similar population growth (Chatrath et al. 2007). India has previously imported wheat to maintain government buffer stocks when there has been low domestic wheat procurement (Dorosh 2009). Australia has exported wheat to India with a recent shipment of 1.2 kilo tonnes of wheat in bags and containers in October 2010 (ABARE 2011b). As India's economy continues to grow and wheat production stabilises, there may be potential for future wheat imports from Australia to maintain food security; especially as the Indian population consumes more wheat based foods (MacAulay 2010).

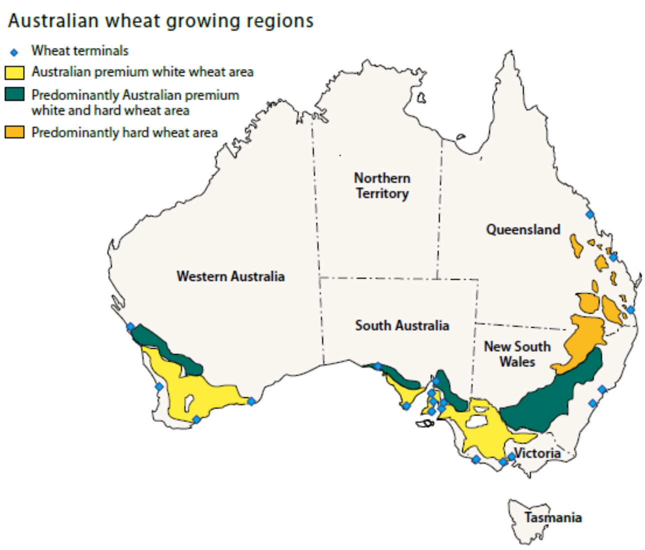
1.2 The wheat industry of Australia

Wheat production in Australia is an integral part of the Australian economy and has continued to experience long term growth since the 1960's. Wheat is Australia's largest crop and is grown as a winter cereal with 27.8 million tonnes produced in 2010/ 11 (ABARE 2011a). Due to comparatively small domestic requirements, Australia is predominantly a wheat exporting country with an average of 67% of grain including flour exported from 2005 to 2011 (ABARE 2011a). In 2010/ 11 Australia exported 66.8% of wheat produced, worth AUD $5.5 billion dollars (ABARE 2011a). Accordingly, new market opportunities for Australian grain are continually being identified and are actively sought after. The Organisation for Economic Cooperation and Development (OECD) and the Food and Agriculture Organisation of the United Nations (FAO) has forecast that India will become an important growth market for Australian wheat exports in the future (ITS Global 2006).

The global wheat export market is a highly competitive environment. Of the world's wheat production, approximately 19% is traded annually, which was 126 million tonnes in 2010/ 11 (ABARE 2011a). Australia is one of the five largest wheat exporting countries and regions, which includes the United States of America, Canada, the European Union and Argentina. In addition, non-traditional wheat exporting countries including Russia, Kazakhstan and the Ukraine are now emerging as significant competitors. Since July 2008, Australia deregulated the former single desk, bulk wheat export and marketing arrangements of AWB Ltd. Australian wheat exports are now regulated by Wheat Exports Australia (WEA) and there are currently twenty six accredited wheat exporting organisations (Wheat Exports Australia 2008). In 2010/ 11, 57.7% of Australian wheat exports went to eight countries: Indonesia, Korea, Japan, Bangladesh, Malaysia, Iraq, Yemen, and Egypt; with Asia and the Middle East regions major export markets for Australia (ABARE 2011a). To remain internationally competitive, the Australia wheat industry needs to continue to ensure Australian wheat is of high standard; and meets market expectations through innovation and improvement in quality with understanding of the end user.

The wheat growing areas in Australia include the eastern states of Queensland, New South Wales, Victoria and South Australia; and Western Australia, as shown in Figure 1.2. Western Australia (W.A.) has been the largest wheat producing, and also exporting state, over the last decade in Australia. 8.2 million tonnes was produced in 2008/ 09, which was 39% of wheat produced in Australia for that season (ABARE 2011a). Only 5 million tonnes however was produced in 2010/ 11 which was 18% of Australia's wheat production (ABARE 2011a). The classes of wheat most commonly grown in W.A. are Australian Hard (AH), Australian Premium White (APW), Australian Premium White Noodle (APWN), Australian Standard White (ASW), Australian Standard White Noodle (ASWN/ ANW) and Australian Soft (ASFT) (MacAulay 2010; Zaicou-Kunesch et al. 2010). Wheat must meet minimum quality requirements to be segregated into these classes; which includes the ability to make suitable quality end products such as leavened breads, Middle Eastern flat breads, Asian steamed breads and yellow alkaline noodles. It is however, only the APWN grade where wheat is purposely grown in W.A. for the singular end use of white salted noodles and Japanese udon noodles.

Figure 1.2. Australian wheat growing areas.



Source: (ABARE 2012)

The recent development of the APWN class is a notable achievement, principally based on Western Australia's relationship with Japanese flour millers (Wheat Classification Council Australia 2010; Crosbie 1991). Australia has established itself as a well regarded exporter of a range of quality wheat products, targeted for specific and different markets. Furthermore, for the first time 55, 000 tonnes of Western Australian wheat was exported to Saudi Arabia in June 2010 (ABARE 2011b; CBH Group 2010). Attributed to this success was considerable research and development which required demonstration of the ability of W.A. wheat to make quality Arabic flat bread. Published research on the ability of Australian wheat to make the traditional Indian flat unleavened bread chapatti however is not available. The chapatti making abilities of wheat grown in major exporting countries of Canada and the United States of America has been investigated, although not prominently published (Hatcher, Kruger, and Dhaliwal 1997; Dhaliwal et al. 1996). The ability of British wheat to make chapatti has also been explored (Ur-Rehman, Paterson, and Piggott 2007a; Abrol 2003).

Australia has built a reputation as a supplier of quality grain with strong focus on marketing towards end user demand. Australian wheat has not previously been studied for their abilities to make chapatti. Key outcomes of this research will be the generation of new knowledge and greater understanding of wheat quality requirements for chapatti and their affect on chapatti quality. In the future, further strategic marketing of Australian grain to India will be needed in a competitive international wheat market. It will be beneficial to establish long term trade relationships with India and remain their competitive supplier of wheat. Moreover, Australia is one of few major wheat exporters located in the southern hemisphere and its geographical proximity to India is of advantage. Fundamentally providing quality raw material which can meet the customer's requirements by producing quality end products will be essential for export of Australian wheat to India.

1.3 Research aim and objectives

1.3.1 Research aim

The aim of the research was to determine the ability of Australian wheat to make the staple Indian wheat food chapatti; and to investigate wheat quality requirements for chapatti.

1.3.2 Research objectives

To achieve the research aim, the following research objectives were developed.

1. To identify and select suitable quality commercially available Australian wheat varieties for chapatti research.
2. To characterise and compare the quality of Australian and Indian chapatti flours based on physicochemical and rheological properties.
3. To profile the wheat proteins of Australian and Indian chapatti flours using matrix assisted laser desorption/ ionisation time of flight mass spectrometry (MALDI TOF MS).
4. To characterise and compare the quality of chapatti made from Australian and Indian chapatti flours using objective and sensory tests.
5. To identify and determine wheat flour properties which significantly contribute to chapatti quality.

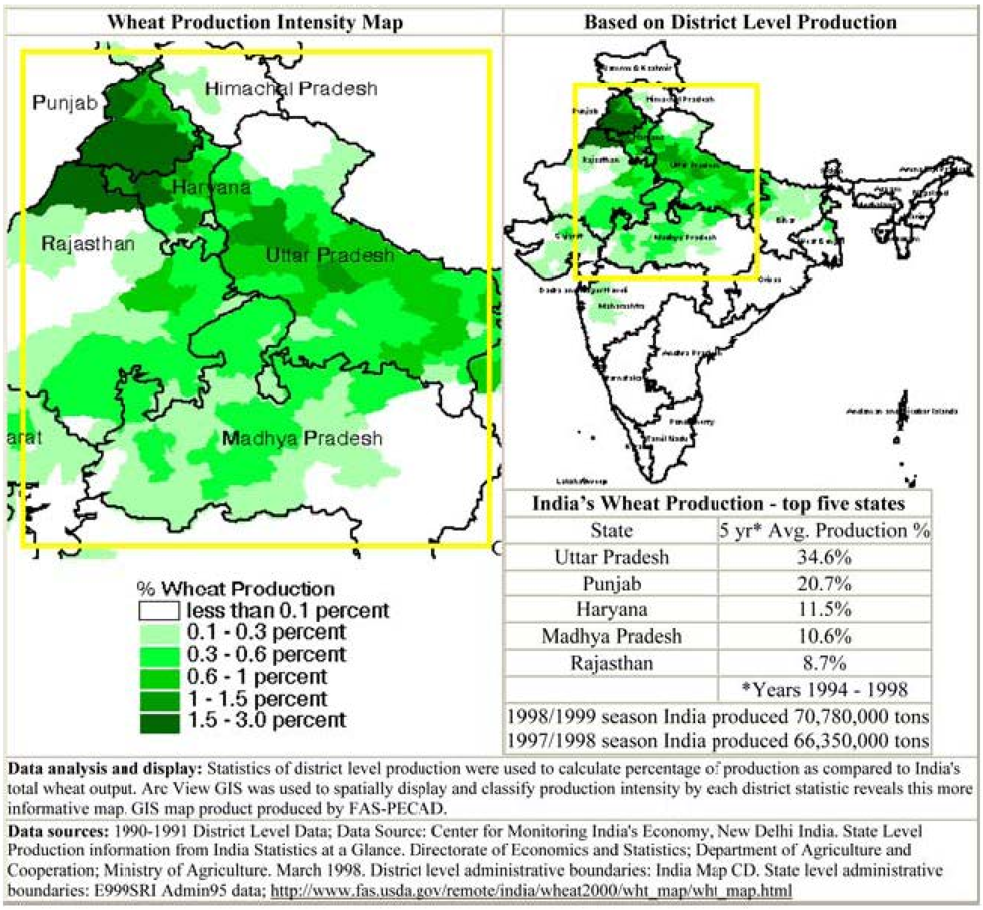
2.0 LITERATURE REVIEW

2.1 Chapatti

Chapatti, with spelling variations of *chapati* and *chapathi*, is a type of flat unleavened bread made from whole wheat flour and water. Origins of chapatti are from ancient times with the word chapatti derived from the word *chappa* meaning ‘flattened’ in the Dravidian language; a language indigenous to India, Pakistan and Sri Lanka (Encyclopædia Britannica 2011). For the people of India and the Indian sub-continent, chapatti is a traditional and staple food. Furthermore, chapatti is also consumed in the Middle East, eastern parts of Africa and by expatriates of these regions in other countries around the world (Ur-Rehman, Paterson, and Piggott 2007b; Dhaliwal et al. 1996). There are also traditional product variants of chapatti and these include tandoori roti, paratha, phulka and puri (Yadav et al. 2008a; Haridas Rao and Manohar 2003).

In India, chapattis are generally consumed twice a day and are traditionally prepared fresh at mealtimes as they stale rapidly (Prabhasankar and Manohar 2002; Haridas Rao, Leelavathi, and Shurpalekar 1986b). To consume, chapattis are usually torn into smaller pieces and made into a scoop with the thumb and forefingers to eat with meat, vegetable or pulse curry dishes (Shaikh, Ghodke, and Ananthanarayan 2007; Venkateswara Rao et al. 1986). Within India, higher consumption of wheat based foods is in the north western regions of the country; these are the areas where wheat is mainly produced, see Figure 2.1 (Joshi et al. 2007). Chapattis substantially contribute to the daily nutritional requirements of the Indian people and for some it is the main component of their daily diet (Yadav et al. 2008b). Therefore it is not an uncommon practice to blend wheat flour with other flours like millet, sorghum, chickpea and corn (Sidhu 1995). Two rationales for preparing composite flour chapattis are to reduce cost by blending and to increase the nutritional value of chapatti.

Figure 2.1. Wheat producing states and areas in India.



Source: (Joshi et al. 2007)

2.1.1 Whole wheat flour

Traditionally, chapattis are made from stone milled whole wheat flour referred to as *atta*. Wheat is procured by Indian villagers and may be stored for up to a year at a time. The wheat is usually manually hand cleaned, washed and dried, before being taken to the local village stone mill for milling. Stone mills are the common type of mill prevalent in villages throughout India and are locally known as a *chakki* (Gujral, Singh, and Rosell 2008; Hatcher, Kruger, and Dhaliwal 1997). A typical stone mill consists of two horizontally aligned granite stones of the same diameter, one stationary and the other rotating (Gill, Sodhi, and Kaur 2005). The wheat is crushed between the two stones and the flour collected at the periphery is of approximately 100% extraction; referred to as whole wheat flour or atta. In addition, plate and disk mills have also been referred to as chakkis and the terms ‘plate’ and ‘disk’ mill have been used interchangeably (Prabhasankar and Haridas Rao 2001; Haridas Rao, Leelavathi, and Shurpalekar 1986b).

Whole wheat flour can be characterised as having the original whole grain components; the endosperm, germ and bran, present in the same relative proportions as they are found naturally in the intact caryopsis (Go Grains 2006; U.S. Food and Drug Administration 2006). Stone milled flour of 100% extraction can be used to make chapattis, however it is most common to manually sieve the atta to remove the coarse bran particles. The result is flour of typically 93 to 95% extraction, although in some instances it has been described to range from 85 to 100% extraction (Ur-Rehman, Paterson, and Piggott 2006; Haridas Rao and Manohar 2003). The amount of bran sieved out of the flour is largely determined by personal preference. Whiter colour flour has been noted to be preferred by some consumers as it makes lighter coloured chapatti. Nonetheless, with renewed awareness of the relationship between consumption of whole grain products and positive health outcomes, higher extraction flour, greater than 90%, is more commonly used to make chapatti (Gujral, Singh, and Rosell 2008; Hatcher, Kruger, and Dhaliwal 1997).

In India, it is now common to have both husband and wife working, attributed to the country’s economic growth and thus changes in lifestyle over the last decade. Consequently, the time to have wheat milled at the local stone mill has been reduced. Alternatively, atta can be bought already milled and packaged to make chapatti. The conventional production of commercial chapatti flour involves first pearling the wheat to remove approximately 10% of the bran using a pearling machine (Gujral 2008). The pearled wheat is then milled using a stone mill and all parts of the grain milled are collected as whole wheat flour. A whiter colour chapatti is subsequently produced and this is sometimes preferred by consumers. Limitations with throughput using stone mills and also the emergence of a market for ‘readily available whole wheat flour’, has led the roller flour milling industry to produce commercial chapatti atta flours.

A range of different milling methods have been utilised in research involved with making chapatti. These include using stone mills (Mulla et al. 2010; Gujral and Gaur

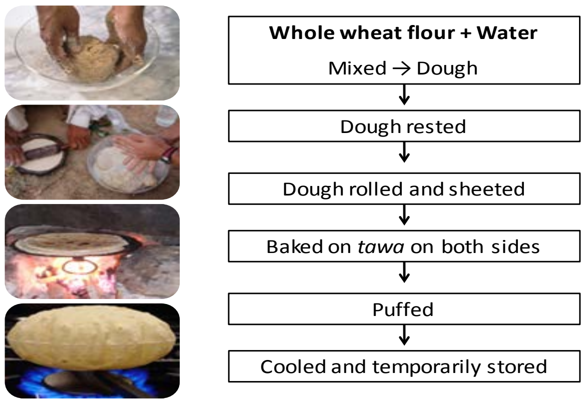
2002), plate mills (Prabhasankar, Manohar, and Gowda 2002), disk mills (Hemalatha et al. 2007; Venkateswara Rao et al. 1986), hammer mills (Srivastava, Prasada Rao, and Haridas Rao 2003; Haridas Rao, Leelavathi, and Shurpalekar 1986b) and roller mills (Hatcher, Kruger, and Dhaliwal 1997; Shurpalekar et al. 1976). The method of milling used to make atta has been shown to have a significant influence on the quality of flour produced and consequently the quality of chapatti. Studies by Sidhu et al. (1988) and Haridas Rao, Leelavathi and Shurpalekar (1989) determined that the highly abrasive action of the stone mill contributed to increased damaged starch, increased water absorption and finer particle size granulation of the resultant flour when compared to other milling techniques. The flour characteristics described are important quality requirements for chapatti and have been significantly correlated to chapatti textural attributes (Prabhasankar, Manohar, and Gowda 2002; Haridas Rao, Leelavathi, and Shurpalekar 1989). From a commercial viewpoint, stone milling is not a viable option; instead the roller milling process must be manipulated to produce a suitable quality product. Nonetheless, for small scale production and research, the stone mill or similar plate and disk mills will produce high quality chapatti flour, in comparison to other mill types.

2.1.2 Chapatti making methodology

Traditional chapatti making

The traditional process of making chapatti involves the following key stages, see Figure 2.2. Whole wheat flour and water is mixed by hand to form a dough. The dough is rested for a period which can range from 15 to 60 minutes, usually dependent on the convenience of the housewife (Hemalatha et al. 2007). After resting, the dough is rolled to the desired thickness, usually 1.5 to 3 mm, and shaped which is typically a circle of approximately 150 mm diameter (Sidhu 1995). A cast iron griddle pan or gas heated griddle, referred to as a *tawa*, or sometimes spelt *tava*, is used to cook the chapatti (Dhaliwal et al. 1996; Shurpalekar and Prabhavati 1976). The chapatti is cooked for a short time on one side, flipped and baked on the other side. The chapatti is then gently pressed with a cloth or transferred to a live flame to induce puffing. The water remaining in the chapatti converts to steam and temporarily puffs the chapatti to create two layers. The chapatti is then cooled and may be placed in a cloth lined basket, where the chapattis are stacked and kept warm until consumed (Dhaliwal et al. 1996; Ram and Nigam 1982).

Figure 2.2. Traditional Indian chapatti making process.



The precise formulation and chapatti making method will depend on the regional location, availability of resources, personal preference and technique (Sharma et al. 2004; Dhaliwal et al. 1996; Haridas Rao, Leelavathi, and Shurpalekar 1986b). For example, thicker chapattis are generally observed to be made in the rural areas of India and thinner chapattis in the cities (Chaudhri and Muller 1970). Chapatti may also be made with the addition of salt for taste and sometimes fat which can be incorporated with the flour and water when it is made into dough, or drizzled over the cooked chapatti before serving (Yadav et al. 2008b; Gill, Sodhi, and Kaur 2005).

Laboratory chapatti making methods

Laboratory chapatti making methods described in the literature adhere to the main stages of the traditional process however contain variations. Differences can be noted in the equipment used to prepare and bake chapatti (Yadav et al. 2008b; Haridas Rao, Leelavathi, and Shurpalekar 1986b; Lindell and Walker 1984; Ebeler and Walker 1983); the method of dough preparation, which includes mixing times, dough resting time, dough sheet shape and thickness (Shaikh, Ghodke, and Ananthanarayan 2007; Ur-Rehman et al. 2006; Gujral and Pathak 2002; Shurpalekar and Prabhavati 1976); and variation in baking times and temperatures (Ghodke and Ananthanarayan 2007; Dhaliwal et al. 1996; Bass and Caul 1972). Explanations for discrepancies between methods include regional difference, equipment availability, different research requirements and the lack of a suitable standard method.

Haridas Rao, Leelavathi, and Shurpalekar (1986b) and Dhaliwal et al. (1996) recognised and acknowledged inconsistencies in chapatti making methods reported. Hence they developed standard methods for the laboratory preparation of chapatti. The chapatti test baking method of Haridas Rao, Leelavathi, and Shurpalekar (1986b) has since become the most cited method in chapatti literature and largely accepted as a standard protocol (Safdar et al. 2009; Butt et al. 2007; Revanappa, Bhagwat, and Salimath 2007; Srivastava, Prasada Rao, and Haridas Rao 2003; Prabhasankar 2002; Jagannath, Jayaraman, and Arya 1999). Nonetheless modifications to the method have been made as required to suit the research conducted (Hemalatha et al. 2007; Anjum et al. 2005; Srivastava et al. 2002).

Further review of laboratory chapatti making procedures highlights the need to make adjustments to methodology in the literature. Ghodke and Ananthanarayan (2007) reported that a dough weight of 25 g will make raw chapatti of 15 cm diameter and 2 mm thick. Whereas Shaikh, Ghodke and Ananthanarayan (2007) also describe 30 g of dough will make raw chapatti of the same dimensions; and Gujral, Singh, and Rosell (2008) used 45 g of dough. This suggests that the specific raw materials, and possibly the equipment used, influence the weight of dough required to make chapatti of standard dimensions. In addition, the rapid cooking process means the parameters of cooking time, temperature, the chapatti dimensions, and the weight of the raw dough sheet are highly dependent on each other. Thus when reproducing methods it may be necessary to make changes to the parameters described to suit the particular raw materials and equipment being used. It has also been suggested that there may be inaccuracies in the reporting of cooking temperatures due to inadequate measuring equipment (Gujral 2010). The utilisation of a standard reproducible method within a research project however is appropriate for comparing different chapatti samples.

2.1.3 End product quality

Whole wheat chapatti is ideally creamy in colour with a scattering of light brown spots over the surface. Full puffing of chapatti is also desired so that two distinct layers are formed (Hemalatha et al. 2007; Ur-Rehman, Paterson, and Piggott 2007a; Dhaliwal et al. 1996). The texture of chapatti should be soft and pliable; not tough, leathery or brittle (Gill, Sodhi, and Kaur 2005; Srivastava, Prasada Rao, and Haridas Rao 2003; Gujral and Gaur 2002). Chapattis are generally eaten by tearing into smaller pieces and making into a scoop to eat curry preparations. The ability of the chapatti to tear and fold easily are important quality traits (Akhtar et al. 2008; Shaikh, Ghodke, and Ananthanarayan 2007; Ur-Rehman, Paterson, and Piggott 2007a). In addition, chapatti should have a slight soft chewy texture in the mouth, a sweet wheatish taste and a pleasant wheaty aroma (Srivastava et al. 2002; Dhaliwal et al. 1996; Haridas Rao, Leelavathi, and Shurpalekar 1986b). Collectively these characteristics define chapatti as being of good quality.

To assess the quality attributes of chapatti two approaches are used; sensory and objective evaluation. Sensory assessment is performed by human subjects who evaluate selected properties of the chapatti as directed, typically scoring or using rating scales. Objective tests are also used to quantify specific attributes related to chapatti quality; and utilise equipment and instruments.

Sensory assessment

Trained sensory panels have been identified as the predominant method for evaluating chapatti quality (Revanappa, Bhagwat, and Salimath 2007; Sharma et al. 2004; Srivastava et al. 2002; Dhaliwal et al. 1996; Haridas Rao, Leelavathi, and Shurpalekar 1986b). Trained panellists can effectively quantify and describe chapatti quality traits, and discriminate between samples. Appearance is one of the sensory traits of importance and includes panellists assessing the spotting and background colour of chapatti. Other important characteristics are the textural properties, such as foldability, tearing ease, mouth feel and chewing characteristics. Thus panellists have been required to rate chapatti quality traits including; appearance, texture, pliability, hand feel, tearing ease, stickiness, chewiness, mouth feel, taste, aroma and colour.

Assessment of end product quality using sensory evaluation is highly valuable. To train a sensory panel however involves considerable time and resources. Panellists need to learn how to accurately evaluate each quality characteristic and then demonstrate their ability to produce repeatable and consistent results (Meilgaard, Civille, and Carr 1991). In creating a trained panel it is important to use individuals familiar with the product being assessed and who are sensitive to the quality traits of interest. Disadvantages of conducting sensory panels include the time commitment required and panellist fatigue. Therefore it is not feasible to perform sensory evaluation for a large volume of samples with trained panellists. In the context of wheat breeding programs and the varietal evaluation of new lines, it is not viable to conduct end product baking tests and sensory assessments until the latter elite germplasm screening stages. Thus in consideration of these issues, the use of one or two trained assessors can be valuable to provide preliminary insight into chapatti quality of samples. However, there are limitations with using only one or two trained sensory assessors such as the use of data collected is restricted due to the small sample size; and if the assessors are also involved in the research and have prior knowledge about the samples being evaluated this may influence the findings. To minimise these biases techniques such as randomisation of samples and coding of samples to de-identify them can be used.

Objective quality measurement

End product quality attributes can alternatively be assessed and quantified using objective testing procedures, thus allowing larger number of samples to be evaluated. The application of objective test protocols to evaluate the quality characteristics of chapatti has become more prevalent in the last decade. Technological advances and greater access to instrumentation widely used in cereal and baking research has contributed to the increased use. Chapatti quality attributes, objectively measured, include puffed height, textural characteristics and chapatti colour (Srivastava, Prasada Rao, and Haridas Rao 2003; Dhaliwal et al. 1996). Inarguably, objective tests cannot provide as complete descriptive information about end products as sensory analysis. However, standardised, objective and reproducible quality testing protocols are important, allowing comparisons to be made between samples, removing reliance on sensory panels for discrimination.

The full and complete puffing of chapatti has been notably described as an important quality index. Puffing occurs when the moisture remaining in the chapatti is converted to steam causing the chapatti to temporarily puff. Full and complete puffing is desired as it creates two layers, a defining characteristic of chapatti (Hemalatha et al. 2007). Flour water absorption, protein quantity and quality, and physical cracks and tears, are some significant factors affecting the ability of chapatti to fully puff (Srivastava, Prasada Rao, and Haridas Rao 2003; Prabhasankar, Manohar, and Gowda 2002; Ram and Nigam 1982). The puffed height can be measured using a vertical measuring rule, and needs to be measured immediately after puffing is complete (Haridas Rao, Leelavathi, and Shurpalekar 1986b).

Bake loss refers to the difference in weight of chapatti before and after baking and is expressed as a percentage. The loss of moisture from chapatti during baking is the predominant attribute being quantified. While reference to bake loss or moisture loss is limited, it may have application as a simple measure of quality (Gujral, Haros, and Rosell 2004; Ram and Nigam 1982). Bake loss may have correlations to particular flour or chapatti quality traits such as water absorption and puffed height.

Textural attributes can be described to include hand feel, tearing ease, pliability and chewiness; and are important determinants of chapatti quality. The development of objective testing protocols to assess chapatti texture can be seen in the literature as suitable instrumentation also evolved. Currently, the predominant instruments used to measure properties of chapatti texture are multi-functional instruments like the Texture Analyser (Stable Microsystems) and the Universal Testing Machine (UTM) (Instron). These instruments are used extensively due to their capability of measuring a range of different textural characteristics, of a wide range of end products, on the one machine. In addition, the analysis is highly accurate, reproducible and a number of test parameters can be measured in the one test (Bourne 2002).

Venkateswara Rao et al. (1986) reported one of the initial applications of using objective pliability, shear and tearing tests to evaluate chapatti quality. The pliability tester was a device specifically created to measure the pliability of a chapatti strip, by calculating the distance a clamped strip naturally bent (Venkateswara Rao et al.

1986). A paper tearing tester was applied to tear strips of chapatti and a Warner Bratzler shear press was used to measure the force required to shear four layers of chapatti with a conical blade (Venkateswara Rao et al. 1986). Since then, texture analyses measuring actions of extensibility and tearing (Gujral, Singh, and Rosell 2008), cutting (Hemalatha et al. 2010), puncture (Gandhi and Bourne 1988), firmness (Ur-Rehman, Paterson, and Piggott 2007a) and the bite test (Yadav et al. 2009) have been used to evaluate chapatti texture. Variations in the type of test conducted and the texture attachment used is likely due to the availability of equipment. Objective texture analysis is important to include in the assessment of chapatti quality, as it has been shown to significantly differentiate between samples and is suitable for assessing large numbers of samples.

The Warner Bratzler shear press or attachment has been used to measure the shear force required to puncture or, now more commonly, cut strips of chapatti. Venkateswara Rao et al. (1986) reported that Haridas Rao, Leelavathi, and Shurpalekar (1982) used this test during studies on chapatti and the shear values significantly correlated to sensory texture and overall quality ($p<0.001$). Significant correlations ($p<0.05$) were also found by Venkateswara Rao et al. (1986) when comparing the texture of chapatti samples stored in different packaging materials over time. Ram and Nigam (1982) however concluded that the instrument was of limited value in differentiating chapatti samples. Nevertheless, this test is still currently used in chapatti research and has been made accessible with the development of a Warner Bratzler blade attachment for use with the Texture Analyser (Stable Microsystems) and Instron UTM (Hemalatha et al. 2010). However as the Warner Bratzler blade attachment's primary application is for the texture analysis of meat products, it's suitability for cereal and bakery end product texture evaluation is questionable.

Extensibility tests, which can stretch a chapatti strip until it tears, may be more appropriate than cutting tests. The ease of hand tearing chapatti is a measure of quality as it is performed routinely when chapatti is typically consumed. Ghodke and Ananthanarayan (2007) and Gujral and Pathak (2002) have used the textural parameters of extensibility and force-to-tear in studies evaluating the texture of chapattis made with various additives. Greater extensibility of a chapatti strip is indicated by the increased distance it can be extended before tearing; and a lower force to tear indicates softer texture chapatti (Ghodke and Ananthanarayan 2007). Significant differences ($p<0.05$) were found between samples with different additives at varying concentrations; the results were also reproducible (Ghodke and Ananthanarayan 2007; Gujral and Pathak 2002). Thus extensibility and force-to-tear are valuable textural parameters to measure and reliably discriminate between samples; further confirmed in shelf life studies by Gujral, Singh, and Rosell (2008).

The preferred colour of chapatti is described as being creamy and light creamish brown. Chapatti colour however has also been reported as creamy yellow or golden brown; with some consumers preferring a more golden colour (Revanappa, Bhagwat, and Salimath 2007; Hatcher, Kruger, and Dhaliwal 1997). Nonetheless, dark brown or white chapatti is widely recognised as being unacceptable. Whole wheat flour which is used to make chapatti has high bran content. Enzymes, which include polyphenol oxidase (PPO) and peroxidase (POD) are prevalent in wheat bran and cause browning reactions to occur (Hemalatha et al. 2007). Studies by Yadav et al. (2008c) and Hemalatha et al. (2007) determined that there was a significant and positive correlation ($p>0.05$) between chapatti dough colour and chapatti colour. Grain seed coat colour and endosperm colour are predominantly genetically determined traits and thus wheat varieties with high levels of carotenoid pigments and low PPO and POD activities have been suggested as being suitable for chapatti making (Pasha, Anjum, and Morris 2010; Yadav et al. 2008c). Measurement of chapatti colour has been performed using colour measuring instruments which have included different types of colorimeters and spectrophotometers (Yadav et al. 2008c; Shaikh, Ghodke, and Ananthanarayan 2007; Dhaliwal et al. 1996). The reported use of colour measurement to assess chapatti quality however is limited. A shelf life study by Shaikh, Ghodke, and Ananthanarayan (2007) reported no significant changes in the colour of chapatti samples over time; although a study by Dhaliwal et al. (1996) determined significant differences between the colour of chapatti made from wheat of different classes. The measurement of chapatti flour and dough colour may instead be more appropriate.

2.2 Wheat and flour quality

Wheat quality has different meanings for the farmer, the miller, the baker or food manufacturer and the end product consumer. The quality of the end product is largely dependent on the quality of the raw material. Thus end product use typically drives the definition of wheat quality; with different end products having different quality requirements (Khan and Shewry 2009). To define, manage and market grain, wheat quality grade and classification systems have been devised which are particularly important for major wheat exporting countries. After harvest, the quality of grain is assessed and it can be segregated into different grades and classes, defined for making various end products, dependent on meeting receival standard requirements (Productivity Commission 2010). Classification of wheat is primarily determined by the quality attributes of grain colour, grain hardness, grain protein content and dough or protein quality properties (Blakeney et al. 2009).

Wheat quality is influenced by the inherent genetics of the wheat, environmental factors, and the interactions between genotype (G) and environment (E); commonly referred to as G x E (Williams et al. 2008). The effect of these variables on wheat quality varies for different quality traits. Quality characteristics such as growing type, spring or winter; the bran colour, red or white; and kernel texture, hard or soft; are important features used to classify wheat varieties (Khan and Shewry 2009). The aforementioned traits are principally determined by the genotype of a wheat variety. Whereas, the environment has a greater effect on traits such as grain protein content and grain test weight (Blakeney et al. 2009). Thus to take into consideration the impact of environment and G x E interactions on wheat quality; analysis of grain of the same genotype or wheat variety, should be sampled from different growing environments (Williams et al. 2008; Souza et al. 2004).

Physicochemical, rheological and baking tests are routinely performed to assess the quality of wheat varieties and determine their suitability for different end products. Wheat is essentially made up of starch and protein with approximately 65 to 80% of the wheat grain starch, and 8 to 20% protein on a dry weight basis (Pomeranz 1988a). Extensive research has firmly established the importance of both the quantity and quality of starch and protein in determining the suitability of wheat flour for different end products (Pomeranz 1988b). A range of standard chemical and rheological tests can be performed which evaluate the starch and protein properties of wheat.

Wheat starch is largely composed of two polysaccharides amylose and amylopectin. Amylose is essentially a linear macromolecule, composed of glucose molecules linked by α-D-(1→4) bonds and usually makes up 23 to 27% of wheat starch (Cui 2005; Kulp and Ponte 2000). Amylopectin is a highly branched polysaccharide also composed of α-D-glucopyranose residues linked by (1→4) bonds, however branching off this linear polymer are α-(1→6) linkages resulting in a highly branched molecule (Cui 2005; Kulp and Ponte 2000). Standard flour quality tests can be performed to provide information about starch properties. These tests include; the falling number (FN) test, determination of damaged starch content, the flour swelling volume (FSV) test and assessment of starch pasting characteristics using the rapid visco analyser (RVA) (AACC 2000).

Wheat proteins are traditionally classified according to their solubility and extractability as devised by T. B. Osborne and this is still relevant (Wrigley, Bekes, and Bushuk 2006; Weegels, Hamer, and Schofield 1996; Osborne 1907). Based on solubility there are four main types of wheat protein; albumins which are soluble in water, globulins soluble in dilute salt solutions, gliadins soluble in 70% ethanol and glutenins which are soluble in dilute acids or bases (Autran 1993; Pomeranz 1988a). Glutenins include high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS); and together with gliadins (α-, β-, γ-, and ω-gliadins) are the storage proteins of the wheat grain. The gliadin and glutenin proteins are unique in that they are also functional proteins and have a critical role in determining the baking quality of wheat flour.

During dough development, gluten forms from the glutenins and gliadins when mixed with water. The glutenins provide properties of strength and elasticity to the dough and the gliadins confer properties of viscosity and extensibility (Torbica et al. 2007). The dough properties will vary between different flours based on the level of the proteins, and variations in chemical structure and function. These variations are generally referred to as protein quantity and protein quality; respectively. Physical dough tests can provide information about the quality of proteins present in wheat flour and include the farinograph, mixograph, extensigraph and alveograph tests (AACC 2000).

2.2.1 Physical traits

Physical characteristics of the wheat grain, which can be measured, include test weight, kernel weight, grain hardness, screenings, stained kernels and insect damaged grain. Assessment of these traits provides an initial indication of grain and potential processing quality. The physical traits together with other quality attributes are used to segregate and grade wheat.

Test weight

Test weight or hectolitre weight measures the weight of a specific volume of grain and is usually measured with a type of chondrometer. For test weight this is expressed as pounds per bushel and for hectolitre weight as kilograms per hectolitre (kg/ hL) (Khan and Shewry 2009). The test weight value can provide information in relation to grain filling, which is greatly influenced by the environment and indicate flour milling yield potential (Blakeney et al. 2009). In addition, the test weight value can be used for wheat grading purposes, for example to distinguish between milling (greater than 74 kg/ hL) and feed grade (less than 74 kg/ hL) wheat (Blakeney et al. 2009). In regards to chapatti quality, hectolitre weight has been found to have a significant and positive correlation with tearing, puffed height and total chapatti scores ($p<0.05$) and a negative correlation with shear value ($p<0.01$) (Prabhasankar, Manohar, and Gowda 2002; Butt et al. 2001).

Kernel weight

The measurement of kernel weight provides descriptive information about grain quality in regards to grain size. Knowledge about grain size in conjunction with test weight is helpful for inferring the potential milling performance and flour yield of grain samples. Heavier kernels have a greater percentage of endosperm than lighter kernels (Khan and Shewry 2009). A common method to determine kernel weight is to count and then weigh 1000 individual clean intact kernels referred to as thousand kernel weight (TKW). An alternate method to obtain kernel weight data is by using the Single Kernel Characterisation System (SKCS) (Perten Instruments) which measures grain parameters of weight, diameter, moisture and hardness index on a sample of 300 individual kernels per test (Osborne and Anderssen 2003).

Grain hardness

Grain hardness describes the texture of the wheat kernel and is defined as the physical resistance to deformation or shear force (Khan and Shewry 2009; Turnbull and Rahman 2002). Kernel texture is broadly described as either hard or soft and is used as a fundamental means to classify wheat. Grain hardness is largely genetically determined and results from the packing of starch granules, protein matrix and air cavities in the endosperm (Khan and Shewry 2009). Thus grain hardness influences milling performance and consequently the flour yield, damaged starch content and particle size index (PSI) of wheat flour (Khan and Shewry 2009). The SKCS instrument was developed to measure the hardness index (HI) of wheat and is calibrated against the reference method, the PSI test (Osborne and Anderssen 2003). The main advantage of performing SKCS testing is the simple, rapid and clean analysis, it requires only 300 grains per test, and additionally profiles each individual grain.

The particle size index (PSI) test is the reference method used to measure grain hardness. Determination of PSI involves milling ten grams wheat using standard grinding conditions. The milled wheat is then placed on a sieve with mesh of set aperture and mechanically sifted for two minutes. The weight of material passing through the sieve is used to calculate the PSI. The softer the kernel texture the higher the PSI value as finer flour is produced and thus more milled material passes through the sieve. Near infrared reflectance (NIR) and laser instruments however are now also widely utilised for characterising the PSI of flours (Kulp and Ponte 2000).

Wheat of medium hardness is commonly referred to as being suitable for chapatti (Nagarajan 2006; Haridas Rao, Leelavathi, and Shurpalekar 1986b). Hard wheat rather than soft is needed for chapatti making due to the high damaged starch, high water absorption and protein properties associated with hard wheat types (Prabhasankar and Manohar 2002). Interestingly, a finer particle size flour has been significantly correlated ($p>0.05$) to more extensible chapatti texture and related to softer chapatti (Gujral and Pathak 2002; Sidhu et al. 1988). Flour particle size generally decreases with decrease in grain hardness.

2.2.2 Chemical traits

A range of standard tests can be performed to characterise the chemical qualities of wheat flour. These tests include but are not limited to the determination of moisture content, ash content, protein content, falling number, flour swelling volume, starch pasting characteristics, damaged starch content and flour colour.

Moisture content

The moisture content of wheat indicates the amount of water present in a sample. The grain moisture content is important for knowing how to appropriately store the grain and for understanding the conditioning requirements prior to milling. The moisture content can be determined using air oven methods on ground wheat or flour (Wheat Marketing Centre Inc. 2008). Rapid tests using electric moisture meters, near infrared transmittance (NIT) or reflectance (NIR) are also widely used but require routine calibration against a reference method (Khan and Shewry 2009).

Ash content

Ash content is a measure of the inorganic minerals present in ground wheat or flour. For whole wheat flour, the ash content can range from approximately 1.0 to 2.0%, and for straight run flour of 70% extraction, the ash content may range from 0.3 to 0.5% (Ghodke 2009; Prabhasankar, Manohar, and Gowda 2002; Cornell and Hoveling 1998). In the wheat grain, the concentration of minerals increases outwards from the centre; thus they are lower in the endosperm and higher in the outer bran layers (Khan and Shewry 2009).

The ash content can also provide an indication of the milling efficiency and the refinement level of the flour. Higher extraction flour will have a greater amount of bran, and hence ash, than lower extraction flour. However low extraction flour with higher than typical ash infers inefficient milling. Flour colour is also affected by the ash content. For chapatti, the ash content has been significantly correlated ($p<0.05$)

to chapatti appearance and shear value. A higher ash content was shown to decrease chapatti lightness (*CIE L**) and change redness (positive *CIE a**) and yellowness (positive *CIE b**). The increase in ash content also contributed to an increase in the shear value making tougher chapatti (Prabhasankar, Manohar, and Gowda 2002; Hatcher, Kruger, and Dhaliwal 1997).

Flour colour

Flour colour influences end product appearance and thus it is an important flour quality trait to measure. Variables affecting flour colour include flour extraction rate, particle size index, protein content, seed coat colour, carotenoid content, ash content and bran specking (Zhang et al. 2005; Oliver, Blakeney, and Allen 1993). The colour of flour is largely genetically determined and a number of quantitative trait loci (QTL) have been identified as being associated with components of flour colour (Zhang et al. 2009). Colour is commonly quantified using the *CIE* (Commission Internationale de l'Eclairage) *L*, a** and *b** colour space measurement system (Black and Panozzo 2004). *CIE L** quantifies the brightness or lightness (0 black – 100 white); *CIE a** redness (positive value) and greenness (negative value); and *CIE b** yellowness (positive value) and blueness (negative value). A range of colorimeter and spectrophotometer instruments can be used to measure the colour of dry flour and flour water slurries (Khan and Shewry 2009; Black and Panozzo 2004). Specific tests to measure flour water slurry colour include the Agtron test and flour colour graders such as the Kent Jones flour colour grader (Symons and Dexter 1991). Colour is an important aspect of end product appearance and different end products have different quality requirements for colour. Japanese white salted noodles are required to be creamy white, pan bread and Chinese steamed bread to have bright white crumb, and chapatti to be creamy light brown in colour (Black and Panozzo 2004; Mares and Campbell 2001; Dhaliwal et al. 1996).

Protein content

Protein content is a fundamental quality trait used to differentiate wheat into grades, indicate potential end use, and influence the buying and selling price of wheat. The protein content may vary from 6% to 20% although it is more common to range from 8% to 16% for commercially grown wheat (Khan and Shewry 2009). A combination of genetic and environmental factors such as soil type and fertiliser usage affects the protein content. Kjeldahl or combustion nitrogen analysis reference methods are used to determine protein content by measuring nitrogen content (Khan and Shewry 2009; Wheat Marketing Centre Inc. 2008). A factor of 5.7 specific to wheat is used to convert a percentage of nitrogen to a percentage of protein; and the protein content is usually expressed based on a standard grain or flour moisture content (Blakeney et al. 2009). NIR and NIT analyses are commonly used to measure protein content at grain receival locations and in flour mills as fast non-destructive tests; calibrated using reference methods. Protein content affects other parameters including flour water absorption, dough handling and mixing properties, and thus end product quality (Ma et al. 2007). Blending of grain or flour samples to achieve a required protein level is commonly performed to help ensure consistent end product quality.

Falling number

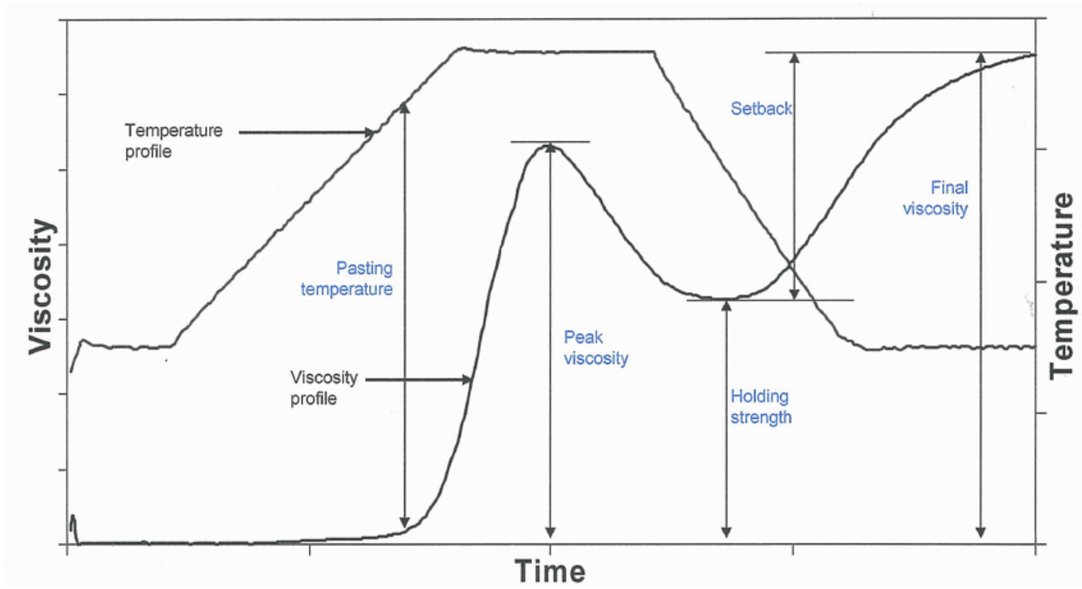
The falling number (FN) test is commonly performed at grain receival to assess the soundness of wheat by measuring α-amylase activity. Rain damage to crops prior to harvest can trigger the event of pre-harvest sprouting and associated increased of α-amylase activity within the grain (Khan and Shewry 2009). The FN is defined as the time in seconds it takes to stir and then allow the stirrer to fall a measured distance through a gelatinised flour suspension undergoing liquefaction by α-amylase (Perten Instruments 2005). A lower FN indicates the stirrer fell faster through a thinner suspension and thus has greater α-amylase activity in comparison to a sample with higher FN. A FN greater than 300 seconds generally indicates sound grain with low α-amylase activity (Wheat Marketing Centre Inc. 2008). Alternative methods to assess α-amylase activity and starch properties include using the rapid visco analyser (RVA) (Perten Instruments) or the Brabender amylograph (Mares and Mrva 2008). Wheat with low FN has been shown to be detrimental to the end product quality of a range of wheat based foods including pan bread, pasta and noodles (Humphreys and Noll 2002). Leelavathi and Haridas Rao (1988) studied the effect germinated wheat had on chapatti quality observing chapatti made from germinated wheat was sweeter in taste but harder in texture; a softer texture however was developed after four days of storage when compared to the control.

Starch pasting characteristics

Starch pasting refers to the events occurring in wheat starch granules when heated in water immediately before, during and after gelatinisation (Zeng et al. 1997). The processes include granular swelling, loss of birefringence, leaching of amylose and the collapse of starch granules, can be measured by changes in viscosity (Crosbie and Ross 2007). The rapid visco analyser (RVA) (Perten Instruments) is one of the most common and more recently developed instruments used to measure starch pasting; an alternative is the Brabender visco-amylograph (Suh and Jane 2003). Studies have compared the two instruments to assess whether they may be used interchangeably, however differences in starch pasting properties have been found (Suh and Jane 2003; Deffenbaugh and Walker 1989).

Standard RVA analysis involves preparing 3.5 g flour and 25.0 mL water in a canister. The slurry is mixed continuously with a paddle while heating to 50 °C, gradually increasing the temperature to 95 °C, holding at this temperature for 2 minutes 30 seconds, before cooling to 50 °C. The viscosity of the sample is recorded throughout the test and a RVA curve is produced. The viscosity parameters: peak viscosity, holding strength, final viscosity, breakdown and setback can be obtained from the RVA curve, see Figure 2.3. The peak viscosity is the maximum viscosity obtained during the heating phase and occurs when the level of granular swelling equals the rupture and breakdown of starch granules; holding strength is the minimum viscosity after the peak viscosity; final viscosity is the maximum viscosity at the end of the test; breakdown is the difference between peak viscosity and holding strength; and setback is the difference between final and peak viscosities (Crosbie and Ross 2007). Variation in pasting properties has been partially attributed to α-amylase activity. The use of appropriate inhibitors which include silver nitrate and β-cyclodextrin can eliminate this variable (Khan and Shewry 2009; Crosbie et al. 1999). There has been limited research investigating the relationship between starch pasting properties and chapatti quality. However a study by Prabhasankar, Manohar and Gowda (2002) determined significant negative correlations with hot and cold paste viscosities and tearing strength and total chapatti quality scores ($p<0.05$).

Figure 2.3. Standard RVA pasting curve.



Source: (Crosbie and Ross 2007)

Flour swelling volume

The flour swelling volume (FSV) test was developed to assess the swelling power of starch granules in wheat flour or isolated starch in a simple and rapid manner. The principle of the flour swelling volume test is based on the swelling of starch granules when they are heated in water above the gelatinisation temperature (Morris et al. 1997). The semi-crystalline structure of the native starch granule begins to melt causing loss of birefringence, breaking of hydrogen bonds between starch molecules and water is imbibed swelling the granule (Goesaert et al. 2005). The FSV test involves preparing a flour or starch and water suspension in a test tube. The test tube is incubated and turned in a water bath at 92.5 °C for 30 minutes. After the incubation time has elapsed the tubes are cooled in ice water and centrifuged. The height of the sediment in the tube is measured (mm) and converted to volume (mL/g). Higher swelling starch samples produce a higher volume gel (Crosbie et al. 1992). Morris et al. (1997) concluded FSV was primarily influenced by genotype and secondly by the environment; thus as a heritable trait it may be used for germplasm selection in wheat breeding programs. The starch swelling properties have been identified as having an important role in noodle quality. Japanese white salted noodles require high swelling starch to provide the soft texture associated with high quality (Crosbie 1991). Conversely, the texture of ramen noodles is firmer and low swelling starch is required to produce quality product (Crosbie et al. 2007; Ross, Quail, and Crosbie 1997). A soft texture is desired for chapatti, and investigation to determine if high swelling starch contributes to producing softer chapatti is needed.

Damaged starch

The damaged starch content is a measure of the damaged starch granules which generally result when milling grain into flour. The shear forces applied during milling physically damage intact starch granules creating exposed starch molecules. The amount of damaged starch occurring is dependent on the type and severity of the grinding and reduction process; and the hardness of the wheat (Boyaci, Williams, and Köksel 2004). The level of starch damage is higher in hard wheat than soft wheat flours (Lin and Czuchajowska 1996). Damaged starch, in comparison to intact starch granules, has an increased water absorption capacity and is more susceptible to enzyme activity particularly from α-amylase (Morgan and Williams 1995). Thus the damaged starch content of flour affects the dough rheology and end product quality.

There are two main types of assays, based on reference methods AACC Approved methods and the Farrand method, used to determine damaged starch content (Morgan and Williams 1995). One type uses enzymatic assays and employs the susceptibility of damaged starch granules to enzyme attack from α-amylase. The resultant products are measured volumetrically or spectrophotometrically (Boyaci, Williams, and Köksel 2004). The other type are iodometric assays which are based on the increased reactivity of iodine with damaged starch granules and the reaction is measured amperometrically or colorimetrically (Morgan and Williams 1995). These assays are widely performed using kits such as the test kits produced by Megazyme International Ireland Ltd., or using instruments like the Chopin SDmatic, as a secondary method, to simply the process and reduce cost. NIR calibrations for predictions of damaged starch are also being developed (Morgan and Williams 1995).

2.2.3 Rheological properties

The assessment of dough rheology provides valuable information about the protein quality and processing performance of wheat flour. The storage proteins, gliadin and glutenin, together with water and mixing, form the protein complex termed gluten (Khan and Shewry 2009). Gluten is principally responsible for the viscoelastic properties of wheat dough; and it has been identified that the viscosity and extensibility is attributed to the gliadin proteins and the strength and elasticity to the glutenin proteins (Wieser 2007). Protein content is strongly correlated to wet gluten content; and both the quantity and the quality of the gluten proteins affect dough rheology (Khan and Shewry 2009; Park et al. 2006; Roels et al. 1993). Hard wheats with high protein content will often produce strong and extensible dough; whereas soft wheats with lower protein content generally form weaker and less extensible dough. Thus, hard wheats with strong and high protein contents are suited to the production of leavened breads; while soft wheats with weaker protein characteristics are used to produce cakes and cookies (Igrejas et al. 2002). To measure dough rheological properties, standard physical dough tests which include the farinograph, mixograph, extensigraph and alveograph are commonly used. New instruments such as the Mixolab® (Chopin Technologies) and doughLAB™ (Perten Instruments) are based on the principles of the aforementioned physical dough tests, and perform similar functions, however are not yet as widely utilised (Collar, Bollain, and Rosell 2007).

The farinograph and mixograph are two widely used torque measuring and recording dough mixers. Both instruments measure the major parameters of flour water absorption, dough development time and tolerance to mixing (Pomeranz 1987). In addition, the farinograph records the properties of dough stability and breakdown (Locken, Loska, and Shuey 1972). The main difference between the two instruments however is the mixing action. A gentle mixing action is performed by the two mixing blades in the farinograph bowl, which is at a constant temperature (Locken, Loska, and Shuey 1972). In comparison, the mixograph has three pins in the bottom bowl and four pins in the top mixing head; designed to replicate high speed commercial mixers in the United States of America (Martinant et al. 1998). The mixograph has a faster and more vigorous mixing action than the farinograph, particularly suited to wheat with stronger protein. Each instrument provides comprehensive analysis of dough properties, although the farinograph provides a more standardised testing procedure than the mixograph (Pomeranz 1987). Furthermore, the farinograph water absorption takes into consideration the protein content, the damaged starch content and flour particle size; in comparison to the mixograph water absorption which is based on the protein content of the flour (Khan and Shewry 2009).

The extensigraph and alveograph measure the overall strength and extensibility of dough made from flour, water and salt using two different approaches. The extensigraph was developed to complement the farinograph; and the farinograph is firstly used to mix and form the dough. The dough is removed from the farinograph bowl, rounded on the extensigraph and shaped into cylinders which are rested in a controlled environmental chamber of the extensigraph (Pomeranz 1987). After resting, a cylinder of dough is stretched by the downward motion of a hook until it breaks (Wheat Marketing Centre Inc. 2008). The main parameters obtained from the extensigraph are resistance to extension, extensibility and area under the curve; quantifying the dough's strength and elastic properties (Grausgruber, Schoggl, and Ruckenbauer 2002). Differing to the uniaxial stretching performed by the extensigraph; the alveograph biaxially stretches dough by blowing air into a flat circular dough piece to form a bubble, which is stretched until it bursts (Pomeranz 1987). P, W and L values are obtained from the curve of the graph produced and interpreted as measures of dough elasticity, strength and extensibility; respectively (Bettge, Rubenthaler, and Pomeranz 1989). One limitation with the alveograph is the fixed water absorption used, problematic for strong hard wheats with high water absorption as they will be under hydrated. Nonetheless, this has since been overcome with the consistograph, a modified alveograph test (Khan and Shewry 2009). The choice of instruments used to measure dough rheological properties may largely depend on the instruments available and secondly the type of wheat being tested.

2.3 Indian wheat classification

The wheat species predominantly grown in India are spring bread wheat varieties of *Triticum aestivum* L. (common bread wheat). In addition, *Triticum dicoccum* (emmer wheat) and *Triticum turgidum* var. durum (durum or macaroni wheat) are also cultivated. The Indian government has established a wheat classification system which differentiates wheat based on quality and end product use. Indian wheat can be segregated into one of five wheat classes; Indian medium hard bread wheat, Indian hard bread wheat, Indian soft bread wheat, Indian durum wheat and Indian dicoccum wheat; see Table 2.1. Wheat which does not meet the requirements of one of these classes is referred to as 'other wheat' (Nagarajan 2004b). The class of wheat suited for the production of chapatti is Indian medium hard bread wheat.

Table 2.1. Classes of Indian wheat.

Wheat class	*Grain description* [1]	*Hectolitre weight (kg/ hL)* [1]	*Protein content (%)* [1]	*Dry gluten (%)*	*Grain moisture (%)*	*End use*
Indian Medium Hard Bread Wheat	Medium grain size and appearance; medium hard grain	> 76	> 10	9	11	Non-fermented flat breads – including *chapatti*
Indian Hard Bread Wheat (Premium Wheat)	Bold and lustrous grain	> 78	> 12	9	11	Fermented and non-fermented breads
Indian Soft Bread Wheat (Biscuit Wheat)	Yellow to white grain; soft grain	75	< 9.5	7	11 - 12	Eastern wheat based foods, biscuits
Indian Durum Wheat	Large and hard grain, vitreous; β-carotene > 5 ppm	> 78	> 12	-	11	Pasta, semolina and extruded products
Indian Dicoccum Wheat	Hard grain, long grain length; β-carotene 5 ppm	> 78	> 13	-	10 - 11	Breakfast cereal, semolina, porridge, extruded products

[1] Abbreviations: (>) greater than and (<) less than.

Source: (Nagarajan 2006; Nagarajan 2004b)

Although a classification system has been devised, it is not one that is currently practiced as there is no set price differential. The driving force for the development of the wheat grading and classification systems arises from India positioning itself as a potential wheat exporting country. India is currently the world's second largest wheat producer and has accumulated considerable wheat buffer stocks from record yielding harvests. Thus, the Indian government has allowed the export of wheat when stocks are too high for the current infrastructure and storage facilities; and when the value of wheat is higher internationally than domestically. To be able to trade in the international wheat market, wheat quality must be defined and include end product characterisation.

2.4 Australian wheat classification

Three main species of wheat are grown in Australia; *Triticum aestivum* L. (common bread wheat), *Triticum turgidum* var. durum (durum or macaroni wheat) and *Triticum compactum* (club wheat). Classification of Australian wheat varieties is currently administered by the Wheat Classification Council of Australia (WCC) formed by the Grains Research and Development Corporation (GRDC). Wheat varieties may be classified into one of eight wheat classes summarised in Table 2.2. However a wheat variety may have a different classification if cultivated in another Australian state due to the differences in growing environment impacting on quality. Well defined and continually evolving wheat quality requirements for each class, contributes to the marketability of Australian wheat by assuring consistent physical quality, processing performance and end product quality.

Table 2.2. Australian wheat classification.

Wheat class	***Grain description***	***Protein content (%)***	***End use***
Australian Prime Hard (APH)	White hard grained wheat; exceptional milling quality; strong and balanced dough properties; high water absorption	13 - 15	Yellow alkaline noodles, ramen noodles, and high protein and high volume breads.
Australian Hard (AH)	Hard grained white wheat; superior milling properties; excellent dough quality; high water absorption	11.5 – 13.5	European style pan and hearth breads, Middle Eastern flat breads, Chinese steamed products, and yellow alkaline noodles.
Australian Premium White (APW)	Hard grained, white multi-purpose wheat; good milling performance; medium to strong dough properties; moderately high to high swelling starch	10 - 12	Middle Eastern and Indian style flat breads, Chinese steamed bread, and various types of noodles.
Australian Premium White Noodle (APWN)	Specific hard grained, white wheat; segregated only in W.A.; good noodle sheet colour; brightness stability	10 - 11.5	White salted noodles, Japanese udon noodles, and instant noodle types.

	and low PPO activity; medium dough strength; high swelling starch		
Australian Premium Durum (APDR)	Hard, vitreous, amber coloured kernels; grain uniformity; high yields of superior quality semolina	13 - 15	Wet and dry pasta products, North African and Middle Eastern products including cous cous, hearth and flat breads.
Australian Soft (ASFT)	White, soft grained wheat; not limited to club wheat varieties; low water absorption; low ash content	7.5 – 9.5	Soft wheat products including biscuits and cakes, steamed bun.
Australian Standard White Noodle (ASWN)	White wheat with relatively soft kernel hardness; high flour pasting properties; low ash	9.5 – 11.5	Udon type noodles, soft wheat products like confectionery and baked foods including sweet biscuits, cakes, pastries and cookies.
Australian Standard White (ASW)	Highly versatile, white wheat; suitable for blending; basic grade wheat	10	Middle Eastern, Indian and Iranian style flat breads, European style breads and rolls, Chinese steamed bread

Source: (Productivity Commission 2010; Wheat Classification Council Australia 2010).

2.5 Quality requirements for chapatti

Whole wheat flour or atta is used to make chapatti and accordingly the quality of atta is of great importance for determining end product quality. Wheat quality requirements for chapatti include medium hard grain with a protein content of 10 - 12% and medium dough strength (Nagarajan 2004a; Prabhasankar, Manohar, and Gowda 2002). In addition, high damaged starch and high water absorption contribute towards making good quality chapatti (Nagarajan 2006).

The importance of damaged starch on the quality of chapatti has been recognised in a number of studies (Ghodke, Ananthanarayan, and Rodrigues 2009; Hemalatha et al. 2007; Ur-Rehman, Paterson, and Piggott 2007a; Prabhasankar, Manohar, and Gowda 2002; Sidhu et al. 1988). High damaged starch content has been significantly and positively correlated to chapatti quality ($p<0.01$) contributing towards a softer textured product (Prabhasankar, Manohar, and Gowda 2002). Furthermore, water absorption and particle size index of flour have been significantly correlated to damaged starch and chapatti quality (Prabhasankar, Manohar, and Gowda 2002; Sidhu et al. 1988). Nonetheless, if the damaged starch content is too high, it can lead to a sticky dough forming which is not desirable for chapatti making (Ghodke, Ananthanarayan, and Rodrigues 2009).

The effect of starch quality in terms of starch swelling, pasting and gelatinisation properties on chapatti quality is not well known. Research has investigated the structural composition of pentosans and chapatti quality (Revanappa, Nandini, and Salimath 2010; Revanappa, Bhagwat, and Salimath 2007; Nandini and Salimath 2001); or has assessed starch gelatinisation using scanning electron microscopy techniques (Hemalatha et al. 2010; Sidhu, Seibel, and Meyer 1990). However the complexity of these analyses makes them unsuitable for routine varietal testing and screening for selected starch properties. Studies by Ur-Rehman, Paterson, and Piggott (2006) and Srivastava et al. (2002) determined higher starch gelatinisation and water retention made more pliable and soft chapatti. Thus relationships between starch swelling, pasting and gelatinisation properties and chapatti quality need further investigation using standard and accessible starch quality tests.

Wheat protein has a critical role in determining the baking potential of wheat flour (Khan and Shewry 2009). The quantity and the quality of protein are both known to have an important role in influencing the end product quality of baked wheat foods and this also applies to chapatti (Safdar et al. 2009; Srivastava, Prasada Rao, and Haridas Rao 2003). The target protein content range for chapatti making is 10 to 12%, although sometimes reported as 10 to 13% (Gupta 2004; Nagarajan 2004a; Austin and Ram 1971). In studies investigating chapatti quality, protein content has been significantly correlated to puffed height ($p<0.05$) (Srivastava, Prasada Rao, and Haridas Rao 2003); and chapatti texture ($p<0.05$) (Manu and Prasada Rao 2008; Ur-Rehman, Paterson, and Piggott 2007a). Conversely it has been concluded that protein content had no significant correlation to chapatti quality attributes (Hemalatha et al. 2007; Prabhasankar, Manohar, and Gowda 2002). The contrasting results indicate that protein content is important, however it may also be protein quality that has a significant role in determining chapatti quality.

A number of studies have confirmed the importance of protein quality on chapatti quality. Manu and Prasada Rao (2008) determined large polymeric proteins (greater than 130 kDa) were correlated to chapatti texture ($p<0.05$); a higher quantity gave harder texture. Hemalatha et al. (2007) found high molecular weight glutenin subunit proteins were significantly correlated to chapatti texture ($p<0.05$) and low molecular weight protein components (20 kDa) significantly correlated to overall chapatti quality ($p<0.05$). In addition, a study by Prabhasankar (2002) reported low gliadin content was suitable for chapatti making. The various findings may be explained by differences in the protein isolation and fractionation methods and the wheat varieties studied. A major limitation with protein isolation techniques are the problems encountered when extracting the protein fractions without disturbing their native state. The incomplete extraction of a protein fraction and the contamination of a protein isolate with other components can occur (Manu and Prasada Rao 2008).

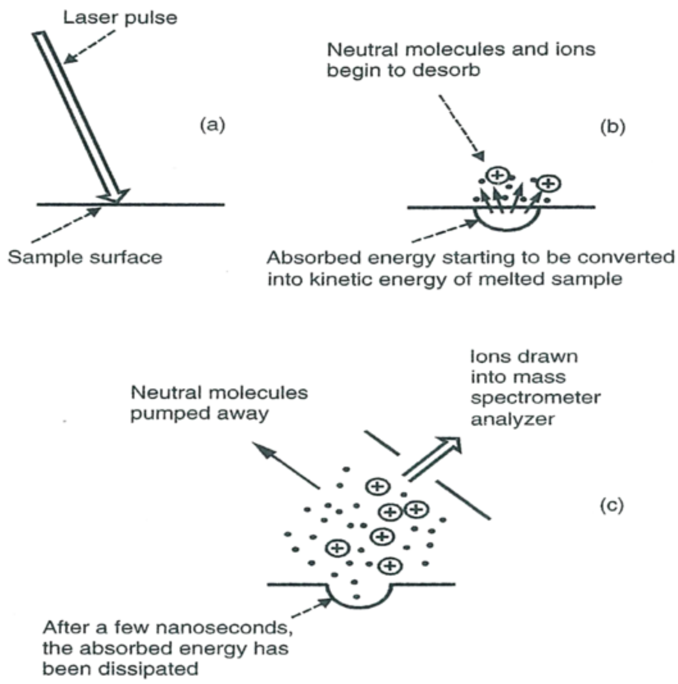
One way to overcome these issues is to use physical dough tests to assess dough properties and rheology. There are however some disadvantages associated with their use, such as the tests being time consuming and requiring a substantial quantity of flour. Nevertheless, the suitability of wheat with medium high or strong protein, or having medium gluten strength for chapatti making is referred to in the literature (Ur-Rehman, Paterson, and Piggott 2007a; Prabhasankar, Manohar, and Gowda 2002). Furthermore, considerable attention has been given to the dough handling properties for chapatti making and these have been described as dough that is non-sticky, and easily sheeted without recoil or shrinkage (Ur-Rehman, Paterson, and Piggott 2007b; Dhaliwal et al. 1996).

2.6 MALDI TOF MS analysis of wheat proteins

Matrix assisted laser desorption/ ionisation time of flight mass spectrometry (MALDI TOF MS) is a powerful tool used in the biological sciences for the analysis of biomolecules. A mass range of 1 to 300 kDa is able to be measured. The most prevalent application for MALDI TOF MS is the analysis of proteins and peptides; with nucleic acids also becoming more widely analysed (Jurinke, Oeth, and van den Boom 2004; Mann, Hendrickson, and Pandey 2001). MALDI TOF MS is considered one of two soft ionisation techniques where gas phase ions are produced, to separate on their ‘mass to charge’ ratio and measured by their time of flight (Jurinke, Oeth, and van den Boom 2004; Storm and Darnhofer-Patel 2003). The greater potential of MALDI TOF MS for protein analysis, in comparison to traditional chromatography and gel electrophoresis techniques, has been shown by the greater accuracy of mass determination, the ability to separate molecules with similar properties, and a reduction in time and labour involved (Bonk and Humeny 2001). MALDI TOF MS provides highly accurate and sensitive detection using high speed analysis, requires only a small sample of 1 picomole to a few femtomoles, and is an automated process (Zhang et al. 2008; Gottlieb et al. 2002).

MALDI TOF MS analysis involves the preparation of a sample or analyte which is embedded into the crystalline structure of a matrix. Preparation of the analyte is critical and usually requires the isolation and purification of a single protein or protein mixture. The analyte and matrix are deposited on a metal substrate, which is a conductive sample support that may hold between one to several hundred analyte spots. Different techniques are used to spot the analyte and matrix including the dried droplet method, thin layer methods, thick layer methods and sandwich methods (Kussmann et al. 1997). The choice of method will depend on the type of matrix and analyte being analysed. The crystallised sample spot, co-crystal, is then irradiated with a pulsed laser beam, typically an ultraviolet (UV) laser with wavelength of 266 or 337 nm. The energy from the laser causes structural decomposition of the co-crystal and generates a particle cloud of ions, which are extracted and accelerated by an electric field, see Figure 2.4. (Jurinke, Oeth, and van den Boom 2004; Mann, Hendrickson, and Pandey 2001).

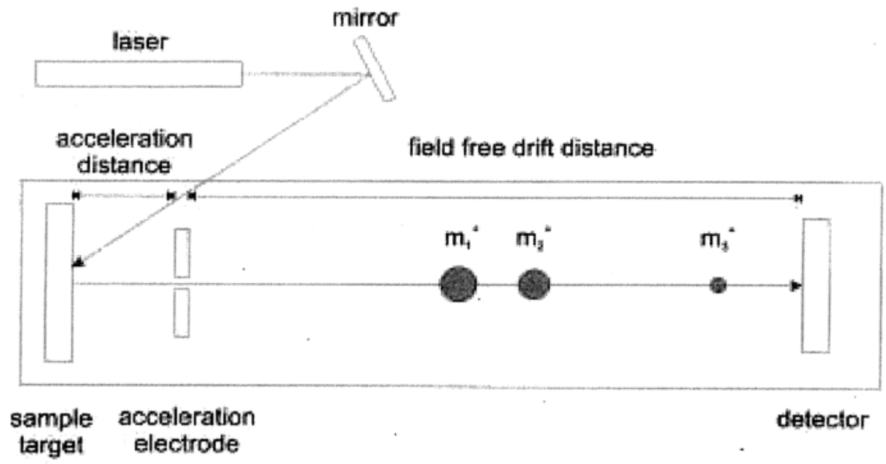
Figure 2.4. Laser desorption and ionisation.



Source: (Herbert and Johnstone 2003)

After acceleration, the predominantly singly charged ions travel through a flight tube which has no electric or magnetic field. Thus the ions pass in a straight line at constant speed to the detector separated by their mass and charge, see Figure 2.5 (Herbert and Johnstone 2003). The mass to charge ratio of the ion is measured by the time of flight, which is the time taken to travel the length of the tube (Bonk and Humeny 2001). Smaller mass ions will travel faster and hence reach the detector sooner than larger mass ions, on the basis of having the same kinetic energy (Storm and Darnhofer-Patel 2003). Numerous pulsed laser shots, sometimes several hundred, are applied to the one sample and the results averaged to produce the final mass spectrum.

Figure 2.5. Schematic diagram of the time of flight pathway.



Source: (Bonk and Humeny 2001)

One of the important aspects of MALDI TOF MS is the use and choice of matrix. Weak organic acids are used as the matrix, with common examples being α-cyano-4-hydroxycinnamic acid (HCCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB) and 2,4,6-trihydroxyacetophenone (THAP) (Kussmann et al. 1997). Different acids will be more suited to particular analytes than others; for example sinapinic acid is a good matrix for proteins (Kussmann et al. 1997). One of the functions of the matrix is to embed and isolate the analyte in its crystalline structure. The matrix also functions to absorb light energy from the laser, and accordingly the matrix should have an absorption band that closely matches the energy of the laser radiation (Herbert and Johnstone 2003). Moreover, as the matrix rapidly absorbs most of the incident energy from the laser, it causes the matrix to vaporise, desorb and ionise quickly. Energy absorbed by the matrix is also passed on to the analyte, which then acts in the same manner (Herbert and Johnstone 2003). One of the advantages of using a matrix is the minimal fragmentation caused to the analyte from laser radiation; instead energy is indirectly absorbed and passed on by the matrix (Herbert and Johnstone 2003; Mann, Hendrickson, and Pandey 2001). Matrix additives such as nitrocellulose may be required or the pH may need to be lowered if the analyte is contaminated with buffers, salts, detergents or denaturants (Kussmann et al. 1997). The concentration of the analyte, the ratio of analyte to matrix, and minimisation of contamination of the analyte, are important factors to optimise to ensure quality mass spectra are produced.

MALDI TOF MS has emerged as a recent technology applied to characterise and investigate structure function relationships of wheat proteins. Considerable emphasis has been on the storage proteins, gliadin and glutenin, due to their functionality and effect on end product use and quality (Qian et al. 2008; Shewry, Halford, and Tatham 1992; Payne 1987). Intensive study of gliadin and glutenin proteins has been previously conducted, elucidating protein components using traditional fractionation techniques. Gel electrophoresis, particularly sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and polyacrylamide gel electrophoresis at acid pH (A-PAGE); and also reverse phase high performance liquid chromatography (RP-HPLC) have most commonly been used (Liu et al. 2009; Weegels, Hamer, and Schofield 1996). MALDI TOF MS however, in comparison to these methods, has the advantages of performing highly accurate, sensitive and rapid analysis. Furthermore, only small samples are required, and proteins and peptides may be analysed in complex mixtures without purification and separation steps (Liu et al. 2009; Zhang et al. 2008). Utilisation of MALDI TOF MS creates the possibility for greater understanding of the structure of wheat proteins, particularly through peptide mapping, and their subsequent functions (Qian et al. 2008). In addition, MALDI TOF MS has potential as a tool for rapid screening of varietal lines for selected proteins in wheat breeding programs (Liu et al. 2009).

The wheat storage proteins consist of monomeric gliadins with intramolecular disulphide bonds, and polymeric glutenins linked by interchain disulphide bonds (Wang et al. 2008). When mixed with water they form the protein matrix gluten and create the unique viscoelastic properties of wheat dough which are associated with quality differences between wheat varieties (Torbica et al. 2007; Gottlieb et al. 2002).

Gliadins contribute properties of viscosity and extensibility to gluten and can be separated into four different fractions, α-, β-, γ- and ω-gliadins, on the basis of their mobility using A-PAGE (Ferranti et al. 2007). The α-, β- and γ-gliadins have molecular weights of 30 to 40 kDa, and the ω-gliadins have molecular weights up to

80 kDa. Gliadins are encoded by genes whose loci are on the short arms of group 1 and 6 chromosomes (Payne 1987). The majority of α- and β-gliadins are encoded at the *Gli-2* loci of chromosome 6, *Gli-A2, Gli-B2, Gli-D2* (Anjum et al. 2007). Whereas, the γ- and ω-gliadins are largely encoded at the *Gli-1* loci of chromosome 1, *Gli-A1, Gli-B1, Gli-D1* (Ferranti et al. 2007). Additionally, minor gliadins have been discovered to be encoded at the *Gli-3*, *Gli-5* and *Gli-6* loci (Metakovsky and Branlard 1998). Gliadins are greatly heterogeneous and highly polymorphic at the *Gli-1* and *Gli-2* loci, with over 100 gliadin alleles identified (Wang et al. 2008; Metakovsky et al. 1997). Moreover, it has been reported that the isolation of 50 gliadin proteins is possible in one analysis; however overlap of bands and presence of protein contaminants are issues with electrophoretic and chromatographic methods (Qian et al. 2008; Mamone et al. 2005; Gottlieb et al. 2002; Wrigley and Shepherd 1974).

Gliadins make up approximately 40 to 50% of the gluten proteins with the precise ratio of gliadins to glutenins affecting the viscoelastic properties of dough (Wrigley, Bekes, and Bushuk 2006; Gale 2005). In addition, correlations between specific gliadin proteins, dough strength and bread making quality have been identified (Wang et al. 2008; Cornish et al. 2001; Metakovsky et al. 1997). Characterisation of gliadins has also been performed extensively to identify wheat varieties and investigate genetic diversity among wheat collections (Qian et al. 2008; Ram et al. 2005a; Cornish et al. 2001). MALDI TOF MS has the potential to characterise gliadins with greater precision and resolution than traditional separation techniques and may help to further explore correlations between particular gliadins and wheat quality (Mamone et al. 2005). Faster wheat variety identification may also become an application.

Glutenin is composed of low molecular weight glutenin subunits (LMW-GS), mass range 30 to 45 kDa, and high molecular weight glutenin subunits (HMW-GS) with a mass range of 65 to 90 kDa (Shewry, Tatham, and Lazzeri 1997). The glutenin proteins provide strength and elastic properties to dough which is important for baked leavened products. The LMW-GS are more prevalent than the HMW-GS, being approximately three times greater in amount (Wrigley, Bekes, and Bushuk

2006; Shewry, Tatham, and Lazzeri 1997). Due to their greater complexity, heterogeneity and overlap with other polypeptides in SDS-PAGE analysis; the LMW-GS are not as well characterised as the HMW-GS (Ferrante et al. 2006; Wrigley, Bekes, and Bushuk 2006). The use of 2D gel electrophoresis has provided improved resolution of LMW-GS, however they remain complex proteins to analyse (Wrigley, Bekes, and Bushuk 2006). One of the contributing factors is the similarity in size and structure of LMW-GS and γ-gliadins; nonetheless they maintain the functionality of glutenins (Liu et al. 2009; Ferrante et al. 2006).

The genes controlling the LMW-GS are located at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosomes 1A, 1B and 1D (Wrigley, Bekes, and Bushuk 2006; Payne 1987). There is considerable allelic variation at the LWW-GS loci and different LMW-GS alleles have been significantly correlated to dough properties (He et al. 2005; Cornish et al. 2001; Gupta et al. 1994). While it is widely acknowledged that the HMW-GS have a greater influence on dough rheology than the LMW-GS, much of this work has focused on dough quality for leavened pan bread (He et al. 2005; Payne et al. 1987). The relative importance of the HMW and LMW glutenin subunits for other wheat end products still needs further investigation. Greater clarification and understanding of the structure and function of LMW-GS is therefore required as they are an important contributing factor to end product use and quality.

The HMW-GS are encoded at the *Glu-1* loci on the long arms of chromosomes 1A, 1B, and 1D; and at each loci an x-type and y-type subunit are encoded. Not all genes however are expressed, with subunits 1Ax and 1By only expressed in some bread wheat cultivars, and 1Ay is largely absent (Shewry, Halford, and Tatham 1992; Payne et al. 1987). The presence of particular HMW-GS has a significant effect on dough rheology and consequently end product use and quality, as initially characterised by Payne et al. (1987). HMW-GS 5+10 are well known to have positive associations with good bread making quality and the combination of HMW-GS 2+12 associated with poor pan bread quality (Gianibelli et al. 2001; Shewry, Tatham, and Lazzeri 1997). Typical wheat cultivars will express between three to five HMW-GS and so their role in bread making and effect on wheat end products has been intensively studied (Gianibelli et al. 2001).

Understanding the role specific HMW-GS have in determining the functionality of wheat flour continues to progress with advances in technology. MALDI TOF MS has been shown to have greater sensitivity in distinguishing subunits, in comparison to SDS-PAGE and RP HPLC. HMW-GS such as 2 and 2*; 7 and 7^{OE}; and 8 and 8* can be clearly distinguished, leading to the possibility of discovering novel HMW-GS (Liu et al. 2009). The technique however has the greatest potential and prospective application to rapidly screen wheat lines for HMW-GS in wheat breeding programs. From a single mass spectrum the HMW-GS profile is able to be directly determined, and this has been demonstrated in studies by Liu et al. (2009); Qian et al. (2008); and Dworschak et al. (1998).

Chapatti in contrast to pan bread is made up of more crust than crumb and does not need to maintain a leavened structure. Hence it is likely to have different protein quality requirements. Although the major focus when releasing new wheat varieties in India has been for high yielding, disease resistant cultivars; bread and chapatti making quality are also routinely assessed for acceptability (Joshi et al. 2007; Nagarajan 2006). Investigation of relationships between HMW-GS and chapatti making quality have been conducted, though are not expansive. HMW-GS 1B20 in combination with 1ANull, has been highlighted as being associated with good quality chapatti (Sreeramulu et al. 2004). Interestingly, not many Indian wheat varieties express HMW-GS 20; thus further research is needed to confirm this relationship and to determine whether HMW-GS 1Bx20 and 1By20 are of importance (Nagarajan 2006; Wrigley, Bekes, and Bushuk 2006). In addition, a different study by Sreeramulu and Singh (1994), identified the most common combinations of HMW-GS from 110 commercially released Indian wheat varieties and these were [2*, 2+12, and 7+8] and [2*, 2+12 and 17+18]; further confirmed by Nagarajan (2006). Since chapatti is the most widely consumed wheat food in India, the aforementioned HMW-GS subunit combinations may have influence on chapatti quality.

Nevertheless, research findings in regards to HMW-GS and chapatti quality are conflicting. Das et al. (2006) concluded HMW-GS did not have a significant role in determining chapatti quality. Whereas Srivastava, Prasada Rao, and Haridas Rao

(2003) identified HMW-GS 5+10 correlated to good quality chapatti and HMW-GS 2+12 were associated with poor quality chapatti. Glutenin components of different size and structure were also shown to affect chapatti quality in studies by Prabhasankar (2002), Hemalatha et al. (2007), and Manu and Prasada Rao (2008). The contrasting findings may mean HMW-GS have an influence on chapatti quality but in combination with other proteins.

Gliadins impart properties of viscosity and extensibility to dough, which may contribute towards softer and more pliable chapatti. Siddique, Archana, and Johari (2004) characterised gliadins using SDS-PAGE and RP-HPLC and found correlations between particular gliadins and chapatti quality. In addition, Srivastava et al. (2002) identified the greater film forming abilities of gluten from hard wheat, made softer textured and more pliable chapatti. There has been little research conducted to examine the role LMW-GS, gliadins, albumins and globulins have on chapatti quality. Although not further confirmed, the quantity of LMW-GS proteins has been significantly and positively correlated to chapatti quality (Hemalatha et al. 2007). The albumin and globulin proteins however do not appear to be of relevance as concluded by Prabhasankar (2002). Further research is required to confirm these preliminary associations and to obtain greater understanding of protein quality components and their effect on chapatti quality.

3.0 MATERIALS AND METHODS

3.1 Wheat samples

3.1.1 Australian wheat samples

Sample selection

The National Variety Trials (NVT) grown in the 2009 season in Western Australia (W.A.) were used to source specific Australian wheat varieties for study. Five trials were selected, from the 44 trials grown in W.A., based on differences in the growing environment, the wheat varieties being grown and the predicted quality of the trial. The trials grown at the geographic locations of Binnu, Eradu, Mukinbudin, Munglinup and Williams were chosen. In W.A. environmental regions which give similar crop performance have been grouped together as a zone. There are six different Agzones which have been defined by the Department of Agriculture and Food, Western Australia (DAFWA); as shown in Figure 3.1 (Zaicou-Kunesch et al. 2010). The growing sites Binnu and Eradu are located in Agzone 1, Mukinbudin in Agzone 4, Munglinup in Agzone 6 and Williams in Agzone 3. The main outcome was to obtain samples of selected Australian wheat varieties from a range of different growing environments that would enable evaluation of quality taking into consideration the effect of environment.

Twenty five Australian wheat varieties were selected for study and all were commercially released and available cultivars; see Table 3.1. Grain samples were collected for each of these varieties at all five trial locations with the following exceptions; wheat variety *Peake* was not grown in the Binnu and the Eradu trials, and the wheat varieties *Arrino*, *Yandanooka* and *Zippy* were not grown in the trial at Munglinup. The majority of the wheat varieties were hard wheats, classed as Australian Hard (AH), Australian Premium White (APW) and Australian Standard White (ASW). Several noodle wheats, of class Australian Standard White Noodle (ASWN/ ANW) or Australian Premium Noodle Wheat (APWN) were also included in the selection and these were; *Arrino*, *Binnu*, *Calingiri*, *Fortune* and *Yandanooka*.

Figure 3.1. The six Agzones of Western Australia.

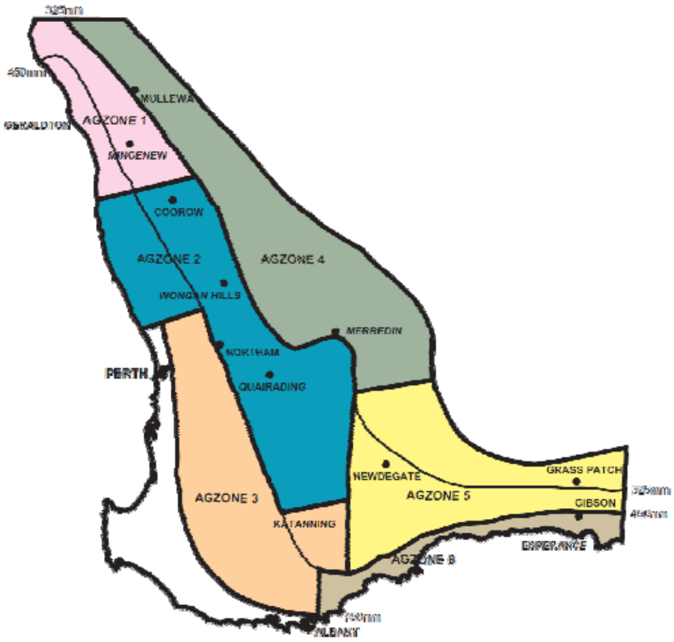


Figure 1 Agzones of Western Australia.

Source: (Zaicou-Kunesch et al. 2010)

Table 3.1. The twenty five Australian wheat varieties selected for evaluation.

Wheat variety	***Class***	***Pedigree***
Arrino	**ASWN**	Complex pedigree(77W:660)/Eradu
Binnu	**ASWN**	(Z1493 660Er)Arrino/(Y89-4034)Eradu*4.VPM.1
Bumper	**ASW**	Express//Pfau/Reeves
Calingiri	**ASWN**	Chino/Kulin//Reeves
Carnamah	**AH**	Bolsena-1CH/WAWHT660 Complex Ped = RAC529/77W:660
Cascades	**AH**	Aroona*3//Tadorna/Inia66
Correll	**APW**	RAC875/Yitpi
EGAWentworth	**APW**	Janz*2/Vulcan
EGA Bonnie Rock	**AH**	Sr9e.3*Warigal..3*Aroona(83Z:1048)/(82W1097)3Ag3.4*Condor ..3*Millewa.3.BodallinBod)
Espada	**APW**	(DH)RAC-875/Krichauff//Excalibur/Kukri/3/RAC-875/Krichauff/4/RAC-875//Excalibur/Kukri
Fang	**APW**	Annuello/2*Stylet
Fortune	**ASWN**	Calingiri/386372//Calingiri/Worrakata[(WqKPWmH*3Ag3Ar)11/9]
Gladius	**APW**	KukriExcalibur.2*RAC875-2.Krichauff/RAC875-22.KukriExcalibur

Guardian	**ASW**	Annuello(VL709)/Krichauff
Kennedy	**FEED**	Hartog/Veery#5
Mace	**AH**	Stylet/2*Wyalkatchem
Magenta	**APW**	Carnamah/Tammin-18
Peake	**APW**	VN-150/VN-715
Tammarin Rock	**AH**	Kalannie/(81Y:970)Skorospelka.4*Lance:3*Bodallin
Westonia	**APW**	Spica.Timgalen(QT2085-20).Tosca(CO1190-203)/(84W127-501)Cranbrook:Jacup*2.Bobwhite
Wyalkatchem	**APW**	Machete/(84W129-504)Gutha.Jacup*2(11Isepton135)Iassul.H567-71
Yandanooka	**ASWN**	Calingiri/1137/(81W:1137)Tammin sib//(WAWHT2029,386443)13IBWSN397(IW:725).Hyden bulk386443
Yitpi	**AH**	(Chamlein*8156)*(Mengavi*Siete Cerros)(Chamlein*8156)*Hron)*(Mengavi*SieteCerros)*Frame/59/1
Young	**APW**	VPM.3*Beulah/Silverstar
Zippy	**APW**	Klasic/Kalannie//Pfau/Reeves

Sample collection

Grain samples of the wheat varieties selected were collected from each of the five NVT sites as the trials were harvested in November and December 2009. The trials were visually inspected prior to harvest, to ensure the correct wheat variety was growing in the corresponding location on the trial layout; see Appendix 1.1 to 1.5. Each wheat variety was grown in triplicate within a trial and each replicate was individually collected in a double labelled calico bag. One labelled tag on the outside of the bag and one labelled tag inside the bag. The grain was directly transferred into the appropriate bag as each wheat variety replicate was harvested and sealed with an elastic ring.

3.1.2 Indian wheat samples

Flour of six Indian wheat varieties was obtained in sufficient quantity for testing from India. The Indian wheat varieties were *C 306*, *HI 1531*, *K 9107*, *PBW 175*, *PBW 343* and *WH 542*. The Indian wheat varieties procured were known to have different chapatti making abilities. It has been reported that Indian wheat cultivars *C*

306, *HI 1531*, *PBW 175* and *K 9107* have good chapatti quality; *PBW 343* average chapatti quality; and *WH 542* poor chapatti quality (Gujral 2009; Srivastava, Prasada Rao, and Haridas Rao 2003). The wheat samples were grown in the states of Haryana and Punjab, and harvested from the 2008/ 09 season. The grain was hand cleaned and stone milled to produce whole wheat flour of 100% extraction which was then sent to Perth, Western Australia. In consideration of Australia's quarantine requirements, it was decided to receive flour rather than grain to enable a more timely delivery of the samples.

3.2 Grain quality assessment

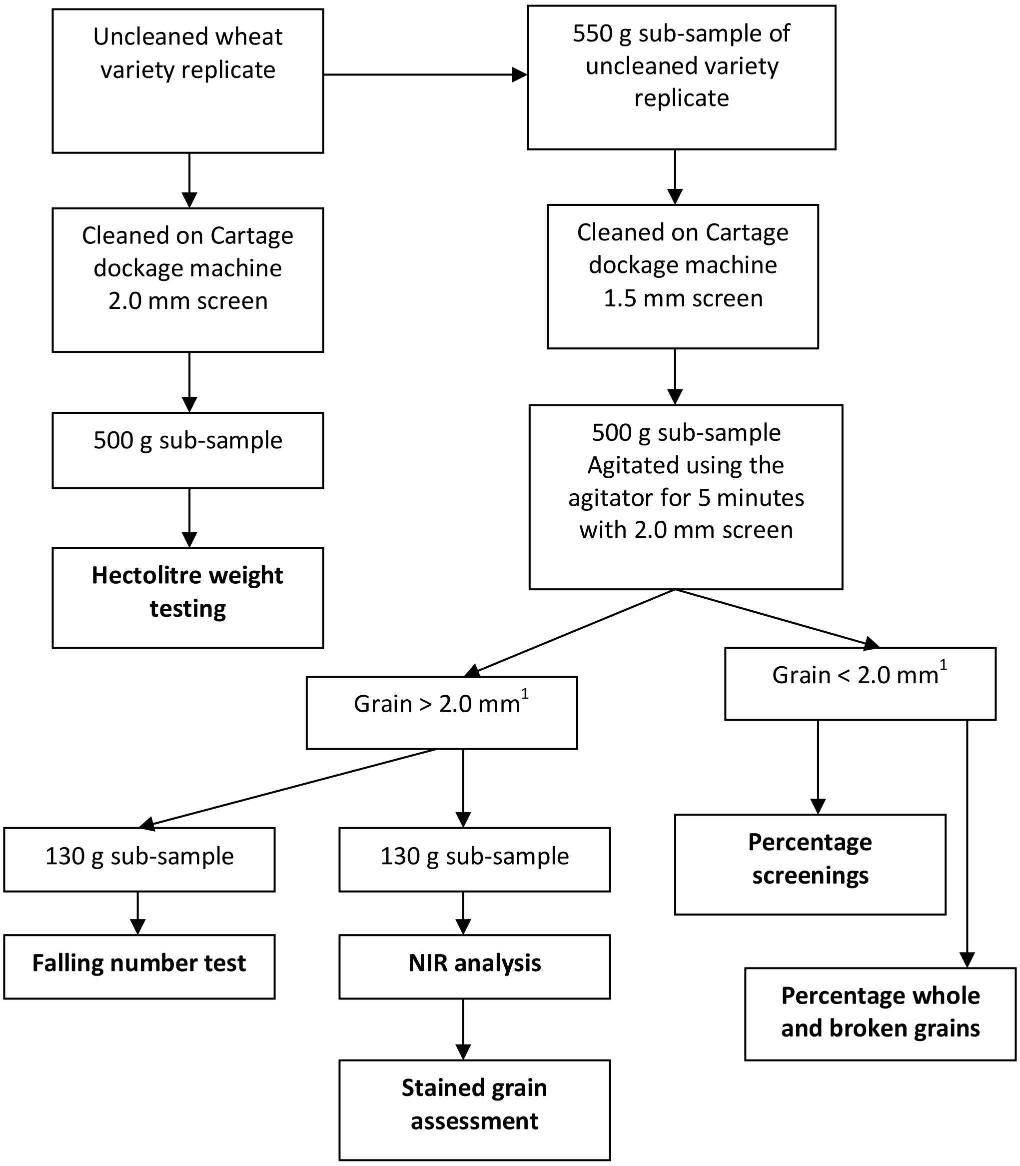
The Australian wheat samples were first visually inspected to confirm that each wheat variety replicate, labelled as A, B, and C, had been correctly collected. The grain quality of each individual wheat variety replicate was then assessed to provide information about the quality of each grain sample and the quality of each trial. Grain quality assessments were conducted to characterise grain quality and are summarised in Table 3.2.

Table 3.2. Grain quality assessment.

		Quality Test	Units
1.	Hectolitre weight		kg/ hL
2.	Percentage screenings – grain less than 2.0 mm		%
3.	Percentage whole and broken grains		%
4.	NIR spectroscopy for predictions of six quality traits:		
	a.	WP – wheat protein	%
	b.	PSI – particle size index	PSI units
	c.	FY – flour yield	%
	d.	b – *CIE b** yellow colour	*CIE b**
	e.	WA – water absorption	%
	f.	fsv – flour swelling volume	mL/ g
5.	Falling number test		seconds
6.	Stained grain assessment		%

The grain samples were prepared for analysis, using the grain quality tests described in Table 3.2, and each variety replicate was processed according to the flow diagram presented in Figure 3.2.

Figure 3.2. Flow diagram of grain sample preparation and testing.



[1] Abbreviation: (>) greater than and (<) less than.

Each wheat variety replicate, A, B and C, from all five trials, was individually cleaned using a Cartage dockage machine fitted with a 2.0 mm screen to remove foreign material, straw, insects and grains less than 2.0 mm. The weight of each replicate was recorded before and after cleaning.

Hectolitre weight was performed as per the Co-operative Bulk Handling (CBH) method used by DAFWA for level three testing. A 500 g sub-sample of grain was taken from the cleaned grain greater than 2.0 mm. The hectolitre weight chondrometer was filled and the metal divider slid through the chondrometer slot. The top section of chondrometer and grain was removed and the lower portion of grain in the chondrometer was weighed. The test was repeated twice to obtain hectolitre weight reported as kg/ hL.

A sub-sample of 550 g of grain was taken from each wheat variety replicate prior to cleaning using sample dividers. The sub-sampled grain was cleaned over a 1.5 mm screen on the Cartage dockage machine. Grain greater than 1.5 mm was collected and stored in a paper bag for further testing; material less than 1.5 mm was weighed and discarded.

To determine the percentage screenings, a 500 g sample of grain was taken from the sample of grain greater than 1.5 mm. The grain was poured into an agitator with a 2.0 mm screen and agitated for 5 minutes. Grain greater than 2.0 mm was returned to the paper bag and the grain less than 2.0 mm was weighed and the weight recorded. The percentage of grain less than 2.0 mm but greater than 1.5 mm was calculated and referred to as the percentage of screenings.

The grain less than 2.0 mm, from the percentage screening analysis, was retained to determine the percentage of whole and broken grains. A sub-sample of the screenings was taken, whose weight was dependent on the value of the percentage of screenings for a sample. A larger weight was taken from a sample with a higher percentage of screenings. The sub-sampled weights ranged from 2 to 5 g. Each sub-sample was tipped into a shallow sided container and the whole intact grains were physically separated from the broken grains with tweezers. The two portions were placed into separate envelopes, weighed and the percentage of whole and broken grains were calculated from the recorded weights.

A sub-sample of 130 g of grain was taken from the cleaned grain greater than 2.0 mm for NIR testing. The sub-sampled grain was placed in a labelled envelope and stapled. NIR spectroscopic analysis was conducted for each wheat variety replicate using a Foss XDS Rapid Content Analyser ™ (FOSS). The sample cell was filled with the sample of grain and scanned. Pre-existing DAFWA calibrations provided predictions for the quality traits of wheat protein, particle size index, flour yield, water absorption, *CIE b** (yellow) colour and flour swelling volume. The NIR spectra were reviewed to check for any errors or out of range results.

An assessment for stained grain was performed on each wheat variety replicate in the Binnu and the Williams trials only; due to the length of time required to perform the evaluation. A visual assessment and identification of the number of tipped, pink and mould grains per 250 kernel sample was conducted. The percentage of tipped, pink and mould grains was then calculated for each wheat variety replicate.

A second sub-sample of 130 g of grain was taken from the cleaned grain greater than 2.0 mm for the falling number test. The falling number test was conducted in duplicate for each of the replicates, A, B and C, of the wheat varieties *Tammarin Rock*, *Westonia* and *Wyalkatchem*. Only selected wheat varieties were tested to eliminate the need to perform falling number tests on all samples collected and reduce testing time. The particular wheat varieties analysed were used as indicators of α-amylase activity for each trial; and were chosen as having increased susceptibility to α-amylase activity of the twenty five wheat varieties studied. The sub-sampled grain was milled using a Clean Mill ™ (Newport Scientific) and the falling number test was performed as per AACC Method 56-81B *Falling Number Determination*, using the Falling Number system (model 1800) (Perten Instruments) (AACC 2000).

After the grain quality assessment was completed, the grain samples were stored in calico bags in a refrigerated environment (0 to 4 °C). Grain samples were re-equilibrated to room temperature (22 to 25 °C) for a minimum of 24 hours prior to further testing. The quality data obtained, allowed informed decisions to be made in regards to the suitability of compositing replicate samples for a wheat variety, and in selecting the trials suitable for study.

3.3 Milling

3.3.1 Wheat sample preparation

The samples collected at two of the five NVT trial site locations were evaluated for this research; the trial at the location of Binnu and the trial at Williams. Grain of the Australian wheat varieties was sub-sampled using sample dividers in preparation for milling. 1.2 kg of grain was sub-sampled from each wheat variety replicate, A, B and C, in the Binnu trial; and 2.0 kg of grain was sub-sampled from each wheat variety replicate B and C in the Williams trial. An insufficient quantity of grain for replicate A of wheat variety *Zippy*, resulted in only replicate B and C being used to maintain uniformity between wheat varieties in the Williams trial. Composite grain samples were created by mixing the sub-sampled grain thoroughly. The grain quality of the replicates was determined to be acceptable, and no significant differences in grain quality between replicates were identified. Therefore, composite grain samples were created to minimise differences in grain quality due to growing conditions and provide sufficient flour for flour and chapatti quality evaluations. In addition, a separate sub-sample of 300 g of grain was taken from the composite grain sample and used for subsequent grain quality tests.

3.3.2 Stone milling

Two identical laboratory stone mills were purchased in India, from Amar Industries by Associate Professor Hardeep Singh Gujral. One was sent to Perth for milling of Australian wheat and the other retained with the Department of Food Science and Technology, Guru Nanak Dev University, Amritsar, India; for milling of Indian wheat samples for this research, see Figure 3.3.

The stones in the mill were set by allowing them to just touch and then turned back enough to just separate them. The feed rate was controlled and the electric current (ampere) monitored during milling to ensure that it did not exceed 10 amperes on the gauge. Fluctuations on the gauge were observed as a result of differences in grain hardness between the wheat varieties milled. Compressed air was used to clean any residual flour from the grain feed and flour exit area after each sample had been milled. The mill stone area was enclosed and not accessible. The set up and milling procedure described was applied to grain samples milled in both Perth, Western Australia and in Amritsar, India. The composite grain samples in the Binnu and the Williams trials were milled on separate occasions. The wheat varieties in each trial were milled in a randomised order. Whole wheat flour of 100% extraction was produced and directly collected into a plastic snap lock bag. The bagged flour was then cooled to room temperature and the bags were separated from each other to maximise air flow around each sample.

Figure 3.3. Laboratory stone mill.

After cooling, the flour was sieved to remove the coarse bran particles and to standardise the milled samples. Whole wheat flour from both the Australian and the Indian milled samples were sieved over an Endecott brass circular sieve, with 600 µm aperture square mesh, using a motorised Endecott sieve shaker (Minor M200, Endecotts Ltd.). 250 g of flour was sieved for 5 minutes, and the flour was collected in a brass pan below the sieve. The sieve was carefully brushed with a soft paint brush to separately remove particles greater than 600 µm and less than 600 µm which adhered to the sieve. The sieving process described was repeated until all flour for a sample had been sieved. The amount of flour able to be sieved at a time was restricted by the dimensions of the Endecott sieve and collection pan. The weights of the separated components were recorded and the sieved flours were sealed in double plastic snap lock bags and placed in plastic air tight containers. The packaged flours were stored in a refrigerated cool room (0 to 4 °C) until required for flour quality testing and chapatti making.

3.4 Wheat and flour quality characterisation

3.4.1 Grain quality

Two grain quality tests were conducted on the composite grain samples of Australian wheat varieties in the Binnu and the Williams trials. One test was performed to determine grain hardness and the other test assessed grain colour.

Grain hardness, defined by a hardness index (HI), was determined using the Single Kernel Characterisation System (SKCS) (model 4100, Perten Instruments). The instrument also measures the grain moisture content, diameter and weight of individual kernels. The grain sample was mixed and a sub-sample of grain taken for testing; any broken kernels or foreign matter were then removed. The sample was loaded into the SKCS machine which analyses 300 kernels per test as described in AACC Method 55-31, *Single-Kernel Characterization System for Wheat Kernel Texture* (AACC 2000). The grain hardness, moisture, diameter and weight results were a mean and standard deviation of the data collected for 300 kernels. The samples within a trial were tested in a randomised order and testing was conducted in triplicate for each wheat variety. The wheat varieties in the Binnu and the Williams trials were assessed on separate occasions.

Grain colour was measured, in *CIE L* a** and *b** colour space units using a Minolta Chroma Meter (model CR 310, Konica Minolta) with the glass light projection tube attachment (CR-A33f, Konica Minolta). The Minolta Chroma Meter was calibrated against the supplied white calibration plate before measuring colour. A 300 g sub-sample of grain was placed in an opaque plastic container. The depth of grain was 2.0 cm, which had been determined to be sufficient to remove any effect of container background on the grain colour reading. The grain samples were checked for foreign contaminants, like insects, grains with their husks intact and broken grains; and these were removed before measuring colour. The Minolta measuring head was pressed into the grain, to a depth of 0.5 cm, and the colour measured. Each wheat variety within a trial was measured in triplicate and the sample testing order was randomised. The wheat varieties in the Binnu and the Williams trials were tested at different times.

3.4.2 Flour quality

The quality of whole wheat flour was assessed by a range of tests which are outlined in Table 3.3. The Australian and the Indian wheat varieties were analysed in triplicate for all tests, except for the farinograph and extensigraph testing which was performed in duplicate. Samples were tested in a randomised order.

Table 3.3 Flour and dough quality tests.

Quality Test	***Method***	***Instruments***
Moisture content	Electrical conduction calibrated with AACC Method 44-15A *Air-Oven Methods*	Marconi moisture meter, model 933C (Marconi Instrument Ltd.)
Ash content	AACC Method 08-01 *Ash – Basic Method*	Thermolyne electric muffle furnace, Series 1000 and 48000 (Laboratory Supply Pty. Ltd.) and crucibles
Protein content	AACC Method 46-30 *Crude Protein – Combustion Method*	LECO model FP-2000 (LECO Corp.)
Falling number test	AACC Method 56-81B *Falling Number Determination*	Falling Number system, model 1800 (Perten Instruments)
Flour swelling volume	AACC Method 56-21 *Flour Swelling Volume*	FSV cooking apparatus and test tubes (DAFWA)
Starch pasting properties	AACC Method 76-21 *General Pasting Method for Wheat or Rye Flour or Starch using the Rapid Visco Analyser* with the addition of 1mM $AgNO_3$ (Crosbie, Chiu, and Ross 2002).	Rapid Visco Analyser (RVA) (Perten Instruments)
Damaged starch content	Assay procedure from Megazyme International Ireland Ltd. for Starch Damage based on AACC Method 76-31 *Determination of Damaged Starch – Spectrophotometric Method*	Novaspec II spectrophotometer (Pharmacia Biotech) and test tubes
Particle size of whole wheat flour	AACC Method 55-40 *Particle Size of Wheat Flour by Laser Instrument*	Malvern Mastersizer 2000 (Malvern Instruments Ltd.)
Farinograph - dough mixing	AACC Method 54-21 *Farinograph Method for Flour*	Brabender farinograph with 50 g bowl (Brabender OHG.)

properties and flour water absorption		
Extensigraph - dough properties of strength and extensibility	AACC Method 54-10 *Extensigraph Method, General*	Brabender extensigraph (Brabender OHG.)

Reference methods: (AACC 2000).

In addition, flour colour was measured using a Minolta Chroma Meter (model CR-400, Konica Minolta) with the light projection tube with 22 mm disc attachment (CR-A33d, Konica Minolta). The colour, *CIE L* a** and *b**, of dry whole wheat flour was measured according to the DAFWA Minolta flour colour method (van Sambeeck, Jefferson, and Pachon 2005). Flour samples were thoroughly mixed and a sub-sample was placed in the granular materials attachment (CR-A50, Konica Minolta) and levelled off. The measuring head of the Minolta Chroma Meter was fitted into the measuring area of the granular materials attachment and the colour was measured and recorded.

The colour of flour and water slurries were also measured and a modified version of the method by Crosbie and Chiu (1998) was used. The flour and water slurries were prepared in a clear plastic Agtron sample cup. 20 g of flour was weighed and 35 mL of water was added; instead of 25 mL as reported by Crosbie and Chiu (1998). The whole wheat flour samples have higher water absorption, in comparison to straight run flour, and thus needed more water to create a slurry consistency. The time period between the water addition to the flour and measurement of colour, was standardised to 1 minute and 10 seconds. During this time 35 mL of distilled water was added to flour, the flour and water were mixed and stirred to create a homogenous slurry, and the colour was then measured through the Agtron sample cup with a RACI standard alabaster tile (Set 12 104) to standardise the background. A Minolta Chroma Meter (Cr-400, Konica Minolta) with glass light projection tube attachment (CR-A33f, Konica Minolta) was used for colour measurement. All samples were prepared and tested in triplicate, and were tested in a randomised order.

3.5 MALDI TOF MS protein quality analysis

3.5.1 Materials

The following solutions, Solution B, Solution B1, Solution B2 and SA solution, were prepared as described below.

- Solution B contained 50 mL 2-propanol, 8 mL tris-HCl pH 8.0 and 42 mL Mili Q water.
- Solution B1 contained 10 mg dithiothreitol (DTT) in 1.0 mL Solution B. The required volume of Solution B1 needs to be prepared just prior to use.
- Solution B2 contained 14 µL 4-vinylpyridine in 1 mL Solution B. The required volume of Solution B2 needs to be prepared just prior to use.
- SA solution contained 10 mg sinapinic acid dissolved in 1 mL of 0.05% TFA and 50% ACN.

3.5.2 HMW and LMW glutenin protein extraction and analysis

First extraction

15 mg of flour for each Australian and Indian wheat variety were weighed in a 1.75 mL tube. 1.0 mL of 70% ethanol was added to all tubes and vortexed continually for 30 minutes at room temperature. The tubes were then centrifuged (5415D Eppendorf centrifuge) at 11, 000 rpm for 5 minutes and the supernatant discarded. 1.0 mL of 55% 2-propanol was added to all tubes and the pellet scraped off the bottom of the tube into suspension. The tubes were then vortexed and incubated at 65 °C for 30 minutes at 900 tr shaking in a Labnet Vortemp 56 (Fisher Biotec). The stages of centrifugation, addition of 1.0 mL of 55% 2-propanol, vortex and incubation at 65 °C for 30 minutes; were repeated another two times. After the last incubation period the tubes were centrifuged at 11, 000 rpm for 5 minutes and the supernatant discarded. 150 µL of solution B1 was added to all tubes which were then vortexed and incubated at 65 °C for 30 minutes at 900 tr shaking. The samples were then centrifuged at 13, 000 rpm for 10 minutes and 60 µL of HMW supernatant was drawn off and put into a new 1.75 mL tube. 40 µL of acetone was added to the HMW tubes and the tubes were put into the freezer overnight to precipitate. The tubes containing the pellet had 90 µL of solution B2 added. The samples were then incubated at 65 °C for 20 minutes at 900 tr shaking and centrifuged at 13, 000 rpm

for 10 minutes. 60 µL of LMW supernatant was put into a new 1.75 mL tube and 240 µL of acetone was added to the LMW tubes which were then put into the freezer overnight to precipitate.

Second extraction

HMW protein extraction

HMW protein samples were taken from the freezer and centrifuged at 13, 000 rpm for 10 minutes. The supernatant was discarded and 90 µL of solution B1 was added to the HMW tubes; they were then incubated at 65 °C for 30 minutes at 900 tr shaking. The samples were centrifuged at 13, 000 rpm for 10 minutes and 60 µL of supernatant was transferred into a new 1.75 mL tube. 40 µL of acetone was added to the supernatant and the HMW samples were put into the freezer to precipitate overnight.

LMW protein extraction

The LMW protein samples were taken from the freezer and centrifuged at 13, 000 rpm for 10 minutes, and the supernatant was discarded. 45 µL of solution B1 was added to the LMW tubes and they were incubated at 65 °C for 30 minutes at 900 tr shaking. 45 µL of solution B2 was then added to the samples and the tubes were incubated at 65 °C for 20 minutes. The samples were centrifuged at 13, 000 rpm for 10 minutes and 60 µL of supernatant was transferred into a new 1.75 mL tube. 240 µL of acetone was added to the supernatant and the LMW protein samples were put into the freezer overnight to precipitate.

MALDI TOF MS plate spotting

The HMW protein samples were taken from the freezer and centrifuged at 13, 000 rpm for 10 minutes. The supernatant was discarded and the tubes air dried for 10 minutes to obtain the final extracted HMW proteins. 60 µL of 0.05% trifluoroacetic acid (TFA) + 50% acetonitrile (ACN) was added to all tubes and then vortexed frequently for 30 minutes to 1 hour. 1.0 µL of the HMW protein samples were transferred into a new 0.6 mL tube. 14.0 µL of SA solution was added and the tubes vortexed. 1.0 µL of the HMW protein and matrix mixture was spotted on a clean MALDI TOF plate and completely air dried before the spotting was repeated.

The LMW protein samples were taken from the freezer and centrifuged at 13, 000 rpm for 10 minutes. The supernatant was discarded and the tubes air dried for 10 minutes to obtain the final extracted LMW proteins. 90 µL of 0.05% TFA + 50% ACN was added to all tubes which were then vortexed frequently for 30 minutes to 1 hour. 1.0 µL of SA solution was spotted on a clean MALDI TOF plate and completely air dried. 1.0 µL of LMW proteins were then spotted on top of the dried matrix and air dried. 1.0 µL of SA solution was spotted on top of the LMW proteins and air dried.

A Voyager DE-PRO TOF mass spectrometer (Applied Biosystems®, Life Technologies Australia Pty. Ltd.) was used to perform MALDI-TOF mass spectrometric analysis. The instrument settings were as follows; accelerating voltage 25 kV, delay time 900 ns, laser intensity 2500, grid voltage 92%, guide wire 0.3%, HMW mass range 50, 000 to 100, 000 Da and LMW mass range 10, 000 to 50, 000 Da. The mass spectra were acquired in positive linear ion mode and averaged from 50 laser shots x 10 analyses per sample spot to obtain the final mass spectrum. The Bin size was 20 ns and the input bandwidth 20 MHz.

3.5.3 Water soluble protein extraction and analysis

15 mg of flour for each of the Australian and Indian wheat varieties was weighed into separate 1.75 mL centrifuge tubes and 75 µL of Mili Q water was added. A continual vortex was applied to all tubes at room temperature and the tubes were then centrifuged at 10, 000 rpm for 5 minutes. The supernatant for each sample was transferred into a new 0.6 mL tube and frozen. 1.0 µL of the supernatant was transferred into a new centrifuge tube containing 9 µL of SA solution and mixed by vortex. 1.0 µL of the water soluble protein and matrix mixture was spotted on a clean MALDI TOF plate and air dried thoroughly; this was repeated two more times. The instrument settings for the Voyager DE-PRO TOF mass spectrometer were as follows: accelerating voltage 25 kV, delay time 350 ns, 50 laser shots x 10 times per mass spectra and the mass range 2, 000 to 25, 000 Da.

3.5.4 Gliadin protein extraction and analysis

15 mg of flour for each of the Australian and Indian wheat varieties was weighed into a 1.75 mL centrifuge tube and 75 µL of Mili Q water was added. A continual vortex was applied to all tubes at room temperature and the tubes were then centrifuged at 10, 000 rpm for 5 minutes. The supernatant was discarded and 120 µL of 30% ethanol was added to the tubes. The tubes were then continually vortexed for 40 minutes at room temperature and centrifuged at 12, 000 rpm for 5 minutes. The supernatant for each sample was transferred into a new tube. 1.0 µL of the supernatant was transferred into 14 µL of SA solution and mixed by vortex. 1.0 µL of gliadin protein and matrix mixture was spotted on a clean MALDI TOF plate and air dried thoroughly; this was repeated two more times. The instrument settings for the Voyager DE-PRO TOF mass spectrometer were as follows: the accelerating voltage 20 kV, the delay time 600 ns, 50 laser shots x 10 times per mass spectra and the mass range 5, 000 to 50, 000 Da.

3.6 Chapatti making

3.6.1 Determination of flour water absorption for chapatti making

The determination of the optimal water absorption for chapatti making was investigated using several different methods reported in the literature. Firstly, the use of a Henry Simon research water absorption meter was referred to, for determining the optimum water absorption for chapatti making (Haridas Rao, Leelavathi, and Shurpalekar 1986a; Shurpalekar and Prabhavati 1976). Attempts to source a research water absorption meter however were not successful.

Alternatively, Haridas Rao, Leelavathi, and Shurpalekar (1986b) and Shurpalekar and Prabhavati (1976) reported the optimum water absorption as the ‘quantity of water required to get a dough consistency of 450 BU using the farinograph, with the lever setting changed from the normal 1:1 position to 1:3 when a mixing bowl of 50 g capacity is used’. Investigation into the feasibility of performing changes to the Brabender farinograph lever setting however determined that it was not possible due to changes in the equipment housing and technology of operation.

Several studies used the standard Brabender farinograph water absorption at 500 BU for chapatti making; and in some of these studies the flour used to make chapatti was roller milled and of a lower extraction rate than typical whole wheat flour (Ghodke and Ananthanarayan 2007; Ur-Rehman, Paterson, and Piggott 2006; Dhaliwal et al. 1996). Nonetheless, it is known that the standard water absorption determined from the farinograph is not appropriate for chapatti making, as there is excess water to optimum requirements (Gujral 2010). Hence, farinograph experiments, with the 50 g bowl, were conducted which targeted BU values higher than 500 BU. The targeted BU ranged from and included, 500 to 950 BU, with testing at every 50 BU increment. Indian and Australian whole wheat flour samples were used for these water absorption experiments. The conclusion was that while it was possible to select one of these greater target BU values; it was realised that to target a different BU would be time consuming and use considerable flour which was limited in quantity.

Therefore, it was decided to subtract a standard percentage from the optimal water absorption determined from the Brabender farinograph at 500 BU. Further experiments were conducted with Indian and Australian wheat varieties to assess chapatti making when different percentages were subtracted from the farinograph water absorption at 500 BU. The result of these experiments concluded a percentage of 15% was to be subtracted from the water absorption at 500 BU. Each wheat variety tested and made into chapatti, using *3.6.2 Laboratory chapatti making method*, used this procedure. An objective method to determine the optimum water absorption for chapatti making was therefore developed.

A review of *3.6.2 Laboratory chapatti making method*, after chapatti making evaluations of Australian and Indian wheat varieties had been completed; however found that the calculated optimal water absorption may not be the optimum water absorption for all samples. This was based on observations of dough consistency during testing. Thus, when making chapatti using *3.6.3 Second laboratory chapatti making method*; the optimal amount of water was determined subjectively as reported by Gujral, Singh, and Rosell (2008). The aim was to add the maximum amount of water to the flour without creating sticky dough. 200 g of flour was weighed in a bowl and 200 mL of distilled water was measured in a graduated cylinder. 100 mL of water was added to the flour and mixed by hand; graduations of 10 mL of water were then added, followed by further hand mixing to form a dough. As the dough came close to reaching the desired consistency, water was added in smaller quantities using a pipette. A time limit of 10 minutes was set, within which the optimal water absorption for a wheat variety needed to be determined. The result was viscoelastic dough which was not sticky and had good dough sheeting properties for all wheat varieties evaluated.

3.6.2 Laboratory chapatti making method

Background

The *Laboratory chapatti making method* described below was based on a modified version of the laboratory chapatti making method developed by Haridas Rao, Leelavathi, and Shurpalekar (1986b). A review of the literature revealed that the method by Haridas Rao, Leelavathi, and Shurpalekar (1986b) was the most cited chapatti making method; though invariably with minor changes to the methodology. In this research, it was determined that the chapatti test baking method by Haridas Rao, Leelavathi, and Shurpalekar (1986b) with some modifications, was reproducible, objective and suitable for the resources and equipment available. Experiments were conducted to adapt baking and puffing times and temperatures for the customised method. It should be noted that several other laboratory chapatti making methods were trialled including methods by; Yadav et al. (2008c), Gujral and Pathak (2002), Dhaliwal et al. (1996) and Bass and Caul (1972). Quality chapattis however were unable to be successfully reproduced using these methods. It was later understood that the baking temperatures reported may not be entirely accurate, and this was likely to have affected the ability to reproduce chapatti making methods described in the literature (Gujral 2010).

Laboratory chapatti making method

Table 3.4 outlines and describes the laboratory chapatti making method used to make chapatti samples.

The process, from stage four, was repeated another five times to produce six chapattis from one dough sample. The six Indian wheat varieties, the twenty four

Australian wheat varieties from the Binnu trial, and the twenty five Australian wheat varieties from the Williams trial were evaluated using the method described. Each wheat variety was evaluated in triplicate and the samples were tested in a randomised order.

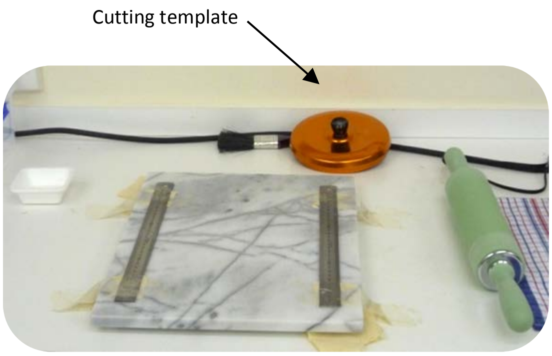
Table 3.4. 3.6.2 Laboratory chapatti making method.

Stage Number	*Stage*	*Description*
1	Weighing	200 g of flour was weighed in a Hobart mixer bowl. Distilled water was weighed into a conical flask and stoppered. The amount of water was pre-determined for each sample.
2	Mixing	Mixing was performed in the Hobart mixer with the dough hook attachment at setting 1, which has a speed of 60 rpm. The flour and water was mixed for 4 minutes.
3	Dough resting	The dough, in the Hobart mixer bowl, was covered and rested for 15 minutes at room temperature (22 °C).
4	Form dough ball	49 g of dough was taken and hand rolled into a ball.
5	Dough sheeting	The dough ball was dusted in flour, 1.5 g, and rolled into a flat circle using a non-stick plastic rolling pin and a marble chopping board with 2.0 mm metal rolling guides on each side, see Figure 3.4. A circular metal cutter with a diameter of 150 mm was used to cut the chapatti dough sheet.
6	Quality testing	The weight and colour of the raw dough sheet was measured and recorded.
7	Baking	The chapatti was placed on a Wenesco electric hot plate (model H0909BA, Wenesco Inc.) with Teflon coating, set at 255 °C. Side one was baked for 70 seconds and side two for 40 seconds, see Figure 3.5 (left image).
8	Puffing	The chapatti, with side one facing up and side two facing down, was transferred into the flat bread oven set at 345 °C and puffed for 25 seconds.
9	Quality testing	After 25 seconds, the puffed height was immediately measured and the degree of puffing visually assessed.
10	Cooling	The chapatti was removed from the oven and placed on a wire rack to cool for 10 minutes at room temperature (22 °C), see Figure 3.5 (right

		image).
	Quality testing	The weight of the cooled chapatti was recorded.
11	Storage	The cooled chapatti was placed in a plastic air tight container.
12	Quality testing	Colour measurements and digital images were taken, followed by texture analyses.

Figure 3.4. Dough sheeting set up and equipment.

3.6.2 Laboratory chapatti making method



3.6.3 Second laboratory chapatti making method

Figure 3.5. Wenesco electric hot plate with Teflon coating (left image); and wire rack for cooling (right image).

3.6.3 Second laboratory chapatti making method

A second laboratory chapatti making method was developed with Associate Professor Hardeep Singh Gujral during his visit to Perth, W.A. in September 2010. The second method was used to validate the results from the first chapatti making and evaluation experiments. Major differences between the two methods included the subjective determination of the optimum water absorption for chapatti making; and alternatively puffing the chapatti on the electric hot plate and not in the flat bread oven. Adjustments to weights, times and temperatures were also made to suit the second chapatti making method.

Based on an analysis of the results from the first chapatti making experiments, eight Australian wheat varieties were selected for further evaluation. The Australian wheat varieties were *Bumper, Espada, Fang, Gladius, Mace, Magenta, Tammarin Rock* and *Yitpi*. Composite flour blends for each wheat variety were created by mixing flour from the Binnu and the Williams trials in an equal amount. The six Indian wheat varieties were also tested. Table 3.5 outlines and describes the stages of the second chapatti making method. Three chapattis were made from one dough sample; and this was conducted in triplicate for each wheat variety and the samples were tested in a randomised order.

Table 3.5. 3.6.3 Second laboratory chapatti making method.

Stage Number	***Stage***	***Description***
1	Weighing	200 g of flour was weighed in a Hobart mixer bowl. Distilled water was weighed into a conical flask and stoppered. The amount of water was pre-determined for each sample.
2	Mixing	Mixing was performed in the Hobart mixer with the dough hook attachment at setting 1, which has a speed of 60 rpm. The flour and water was mixed for 3 minutes and 30 seconds.
3	Dough resting	The dough, in the Hobart mixer bowl, was covered and rested for 15 minutes at room temperature (22 °C).
4	Form dough ball	42 g of dough was taken and hand rolled into a ball.
5	Dough sheeting	The dough ball was dusted in flour, 2.0 g, slightly flattened and rolled into a flat circle using a non-stick plastic rolling pin and a marble chopping board with 2.0 mm metal rolling guides on each side, see Figure 3.4.
6	Quality testing	The weight and colour of the raw dough sheet was then measured and recorded.

7	Baking and Puffing	The chapatti was placed on a Wenesco electric hot plate (model H0909BA, Wenesco Inc.) with Teflon coating, set at 350 °C. Side one was baked for 15 seconds, the chapatti was turned and baked on side two for 30 seconds. During the 30 seconds, the edges of the chapatti were gently pressed with the fingertips, moving around the circumference of the chapatti. The chapatti was then turned again and cooked for a further 20 seconds with no touching and during this time puffing occurred.
8	Quality testing	At the end of the 20 seconds, the puffed height was measured and the degree of puffing was observed and recorded.
9	Cooling	The baked chapatti was then placed on a wire rack and cooled for 15 minutes at room temperature (22 °C).
	Quality testing	The weight of the cooled chapatti was recorded.
10	Storage	The cooled chapatti was placed in a labelled plastic snap lock bag. The chapattis were then left to re-equilibrate for 30 minutes before further evaluation.
11	Quality testing	Colour measurements and digital images were taken, followed by texture analyses.

3.7 Objective quality assessment of chapatti

3.7.1 Percentage bake loss

The weight of the raw chapatti dough sheet and the weight of the chapatti after baking and cooling were recorded for all chapatti made, see Appendix 1.6. The percentage bake loss was then calculated.

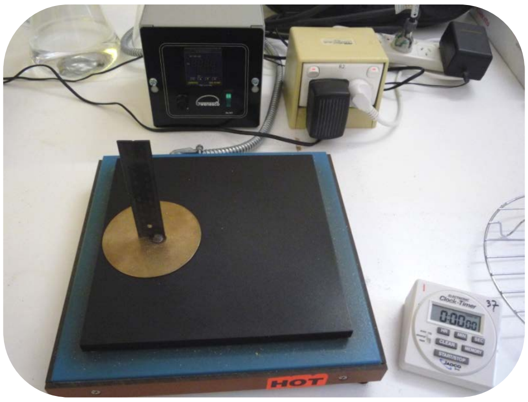
3.7.2 Measurement of puffed height and degree of puffing

The puffed height and the degree of puffing was measured and assessed for all chapattis produced from both laboratory chapatti making methods. Chapatti made using *3.6.2 Laboratory chapatti making method*, were puffed inside an oven for 25 seconds at 345 °C. At the end of the puffing time, the oven door was opened and the puffed height was immediately measured. A vertical metal ruler on a stand had been previously placed next to the chapatti when it was put into the oven. The puffed height measurement was in 0.5 cm increments and a wide flat lifter was used to assist with height measurement; see Figure 3.6. Puffed height was measured from the bottom outer lower surface of the chapatti to the outer top surface of the chapatti. The degree of puffing was also assessed and each chapatti was scored as being either fully puffed, ⅞, ¾, ⅔, ½, ⅓, ¼ or ⅛ puffed, or having only the edges not puffed, see Appendix 1.7. Any cracks, splits or holes observed during puffing were also recorded; and the degree of puffing was assigned a numerical value for statistical analyses.

Figure 3.6. Vertical metal ruler on stand, wide flat lifter for puffed height measurement, and flat bread oven.

A different method of puffing was used for the chapattis made with *3.6.3 Second laboratory chapatti making method*. The chapattis were puffed on an electric hot plate and the puffed height was measured using a vertical metal ruler on a stand. The ruler was placed on the hot plate next to the chapatti and the puffed height was measured to the nearest 0.5 cm; see Figure 3.7. The degree of puffing was also assessed and the chapattis were given a score for puffing, of which the maximum was 40. As a guide, each quarter of the chapatti was allocated a score of 10, and the scores summed to give a final score out of 40. The final score was included in the sensory assessment score.

Figure 3.7. Measurement of puffed height for chapatti made with 3.6.3 Second laboratory chapatti making method.



3.7.3 Texture analyses

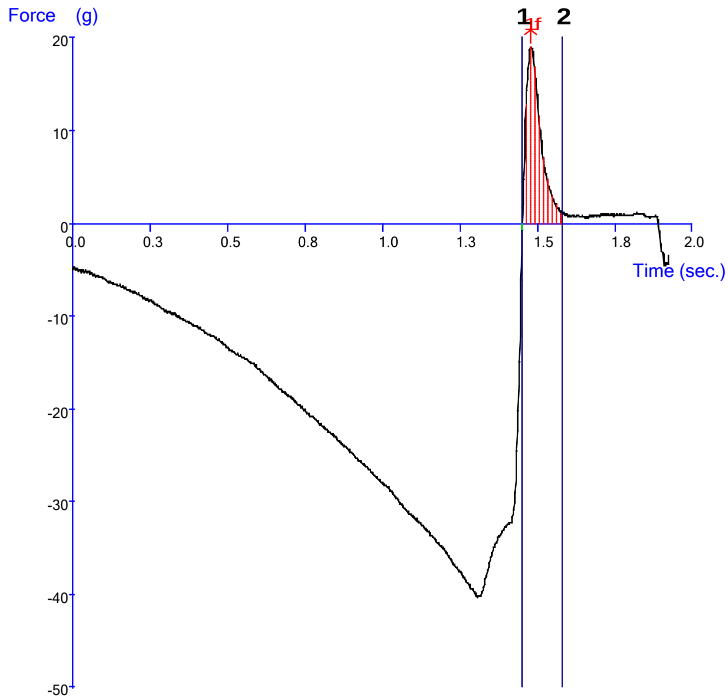
Three tests to assess textural attributes of dough and chapatti were performed using the Texture Analyser (model TA.XT2, Stable Microsystems). The first test measured the dough stickiness of chapatti dough. The second test was the extensibility tearing test of a chapatti strip and the third test was the extensibility puncture test of a whole chapatti. Calibrations for weight and for each of the texture attachments were performed prior to each testing session.

Dough stickiness measurement

Dough stickiness was measured using the Chen-Hoseney dough stickiness cell and 25 mm perspex cylinder probe attachments (Stable Microsystems); see Figure 3.8. 200 g of flour and the optimum amount of distilled water as pre-determined for each sample was used to make dough. The dough was formed using the Hobart mixer with the dough hook attachment, as conducted for chapatti making, described in *3.6.2 Laboratory chapatti making method* and *3.6.3 Second laboratory chapatti making method*. The test was performed as detailed in Appendix 1.8 and an example of the texture analysis results graph is shown in Figure 3.9. Three measurements of dough stickiness were taken per dough sample prepared. Each wheat variety was tested in triplicate and the sample testing order was randomised.

Figure 3.8. Dough stickiness testing set up.

Figure 3.9. Example texture analyser graph for the dough stickiness test.



Extensibility test one - Tearing test

The tearing test was performed on strips of chapatti to measure textural attributes including extensibility through parameters of peak force to tear, distance stretched before tearing, and area under the curve to peak force; which represented the work required to tear the chapatti strip. The tearing test was based on methods by Gujral, Haros, and Rossell (2004) and Gujral and Pathak (2002). Three chapatti strips were prepared for the tearing test and were cut using a rectangular metal template to the dimensions of 50 x 35 mm from one chapatti; see Figure 3.10.

Figure 3.10. Rectangular metal template for cutting chapatti strips.

The tension grip attachment (Stable Microsystems) was used for the tearing test, and cardboard inserts were measured to fit inside the tension grips. The cardboard inserts were used to provide a smooth surface, as opposed to the ridged surface that is standard for the grips; see Figure 3.11. The tension grips were set at 30 mm apart and a chapatti strip was positioned in the grips, with 10 mm of the chapatti strip held by the grips at each end. The grips were tightened until only just secure and the chapatti strip was checked to ensure it was centred and vertically aligned. Side one of the chapatti strip always faced outwards; see Appendix 1.9 for the tearing test settings. A soft brush was used to clean the tension grips and Texture Analyser after each sample was tested. Results for the measurements of peak force to tear, distance stretched before tearing and work required to tear; were obtained from the tearing texture analysis graphs; see Figure 3.12. A macro was set up to identify these measurements from the texture analysis graphs.

Figure 3.11. Tension grip attachment for the tearing test.

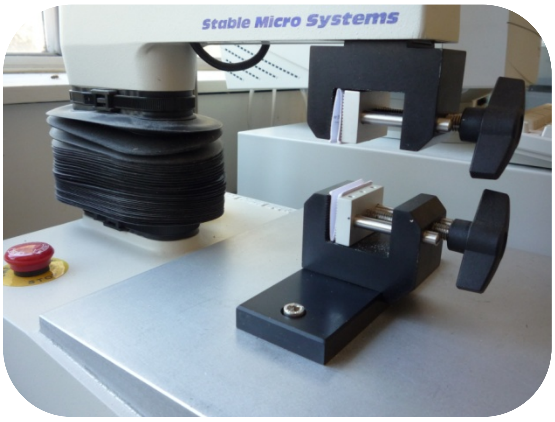
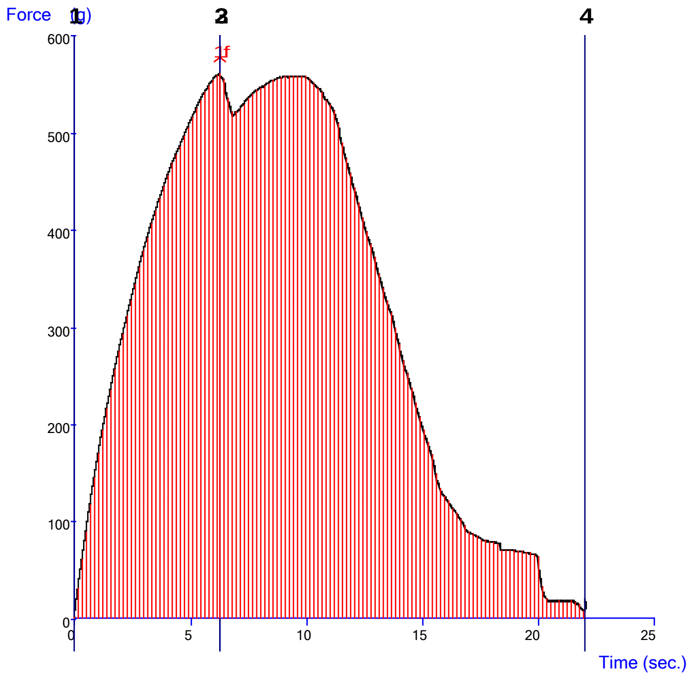


Figure 3.12. Example texture analyser graph for extensibility test one - tearing test.



The chapatti samples tested, for each laboratory chapatti making method, are described in Table 3.6. Each wheat variety was tested in triplicate and followed the randomised chapatti making testing order. Side one was marked on each chapatti strip cut.

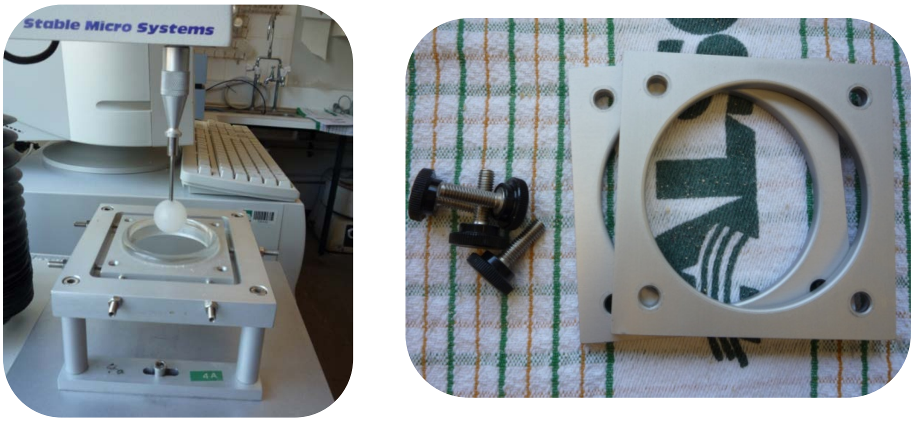
Table 3.6. Number of chapatti and chapatti strips evaluated for the extensibility tearing test.

Laboratory chapatti making method	Number of chapatti made per wheat variety replicate	Number of chapatti used for the tearing test	Number of strips cut per chapatti	Storage conditions until tested
3.6.2 Laboratory chapatti making method	Six	Three chapatti	Three strips cut per chapatti	Plastic air tight container
3.6.3 Second laboratory chapatti making method	Three	One chapatti	Two strips cut per chapatti	Plastic snap lock bag

Extensibility test two - Puncture test

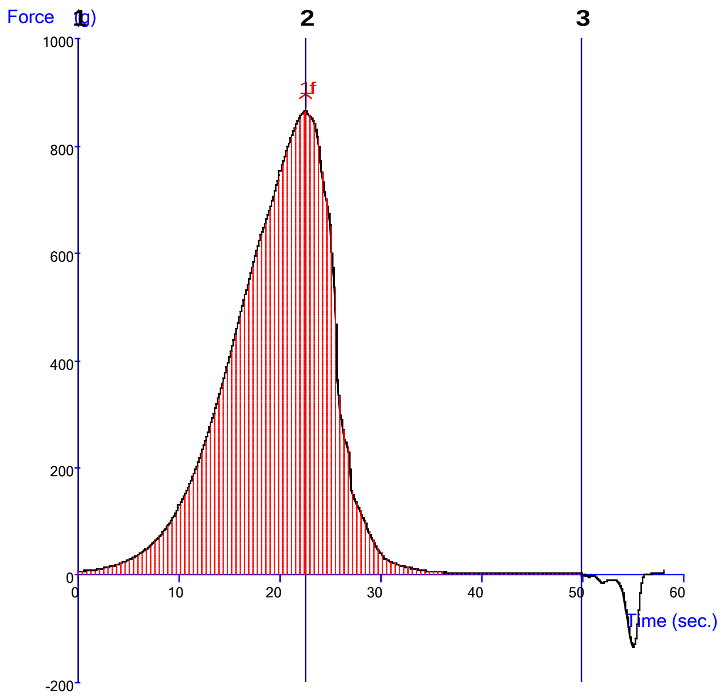
The extensibility puncture test was used to investigate the textural properties of softness, toughness and extensibility of chapatti. An intact whole chapatti was used for the test and it was performed using the tortilla pastry burst rig attachment (Stable Microsystems); see Figure 3.13. The tortilla pastry burst plates were placed centrally on the chapatti, with the four corners of the square plates at the edge of the chapatti being tested. A screw was punched through each of the four corner holes to hold the plates and chapatti in place. The chapatti and plates were then attached, using the screws, to the glass ring plate on the Texture Analyser platform. Side one of the chapatti was placed upwards for all tests. The Texture Analyser platform and glass ring plate were set up and centred on the spherical ball probe which moves through the centre of the chapatti during the test; see Appendix 1.10 for the puncture test settings. A macro was set up to obtain measurements of peak force to puncture, distance stretched before puncturing, and area under the curve to peak force, representing the work required to puncture the chapatti, from the texture analyser graphs; see Figure 3.14.

Figure 3.13. Tortilla pastry burst rig attachment for the puncture test.



All wheat varieties were analysed in triplicate and the sample testing order followed the randomised chapatti making testing order. Three whole chapatti samples were tested per wheat variety replicate made using *3.6.2 Laboratory chapatti making method*. These chapattis were stored in an air tight container until tested. One whole chapatti sample was tested per wheat variety replicate for the chapattis made using *3.6.3 Second laboratory chapatti making method*. The chapattis were stored in a plastic snap lock bag until tested.

Figure 3.14. Example texture analyser graph for extensibility test two – puncture test.



3.7.4 Assessment of appearance

***CIE L* a* b** colour measurement**

The colour of chapatti was measured using a Minolta Chroma Meter (model CR-400, Konica Minolta) with the light projection tube with 22 mm disc attachment (CR-A33d, Konica Minolta). The colour, in *CIE L* a** and *b** colour space units, of the raw chapatti dough sheet was measured at three random locations on each side of the chapatti; see Appendix 1.11. The chapatti background, defined as the area not spotted and browned from baking, was also measured in three random but representative locations on each side of the chapatti; see Appendix 1.12. In addition, the chapatti spot colour was measured on three random but representative brown spots on both sides of the chapatti; see Appendix 1.13. A RACI standard cream tile was used to standardise the background for all chapatti colour measurements, as shown in Figure 3.15. The colour was measured for the six chapattis prepared per wheat variety replicate using the first chapatti making method (Table 3.4) and this was conducted in triplicate for a wheat variety. The chapatti made using *3.6.3 Second laboratory chapatti making method* also had the colour measured as described above, however with the following changes; only two colour measurements were taken on each side of the chapatti instead of three, and the chapatti spot colour was not measured.

Figure 3.15. Chapatti colour measurement set up.

Digital image recording

The overall appearance of each chapatti was recorded using a Panasonic Lumix digital camera (model DMC-FS5, Matsushita Electric Industrial Co. Ltd.). The digital images were captured in a VeriVide light cabinet (model CAC150, VeriVide Ltd.) using two D65 daylight illuminant fluorescent tubes (VeriVide Ltd.). The digital camera was set up on a tripod, in a fixed position, with the camera lens facing down and parallel to the chapatti. Each chapatti was photographed next to a sample label, see Appendix 1.14, and the photographs were taken after the cooling time had elapsed and the colour had been measured. The appearance of each side of the chapatti was different and so both sides were recorded; see Figure 3.16. The chapatti were stored in a plastic air tight container or plastic snap lock bag, depending on the laboratory chapatti making method used, until photographed. The images were saved as Joint Photographic Experts Group (JPEG) image files and used as a reference for the wheat varieties evaluated. Time constraints restricted further analysis of the photographs using image analysis software.

Figure 3.16. Example of the digital images taken of chapatti samples.

Indian wheat variety – *HI 1531*

Side one **Side two**

3.8 Sensory assessment of chapatti

Sensory assessment of chapatti was conducted by Haelee Fenton for the chapatti samples made using *3.6.3 Second laboratory chapatti making method*. One chapatti sample per wheat variety replicate was evaluated and each wheat variety was tested in triplicate. The sensory assessment was conducted thirty minutes after the chapatti had been placed in a plastic snap lock bag. An evaluation of the chapatti quality attributes described in Table 3.7 was completed in the descending order listed. A score for each chapatti quality trait was awarded and the sensory assessment was completed within 10 minutes. The sensory assessment protocol was developed with

Associate Professor Hardeep Singh Gujral during his visit to Perth, W.A. in September 2010.

Table 3.7. Sensory assessment of chapatti quality traits.

Quality Trait	*Description*	*Sensory Assessment Score (100)*
1. Puffing	Assess the degree of puffing, divide chapatti into four quarters and score when puffing on hot plate.	40
2. Colour	White or very dark = lower score. Light brown, yellowish, wheatish yellow, creamish yellow = higher score.	10
3. Appearance	Spotting, a uniform distribution of spots is good.	10
4. Hand feel and pliability	Is it soft, hard, leathery? Hold the chapatti, fold and turn in your hand.	5
5. Hand tearing	Is the tearing uniform or non-uniform? A straight line is better. Rate the ease of tearing, easier is higher score.	5
6. Aroma	Wheatish, pleasant, not charred aroma.	10
7. Taste	Sweetish, pleasant taste, not charred, wheaty.	10
8. Mouthfeel / chewiness	Is it clean, sticky, doughy, hard to chew or soft? Harder, doughy = lower score. Soft, clean, not sticky = higher score.	10

3.9 Statistical analyses

Statistical analyses were performed using GenStat, Fourteenth edition (Version 14.1.0.5943, VSN International Ltd.). The following statistical analyses were conducted; unbalanced analysis of variance (ANOVA), spatial model analysis using irregular grid, correlations, principal component analysis and meta-analysis to generate GGE biplots.

The unbalanced ANOVA allows the general ANOVA model to be fitted to unbalanced data (VSN International Ltd. 2011). The data was unbalanced due to variation in the number of wheat varieties within a data set; and variation in the number of replicate measurements taken for each quality trait. The analysis of variance was carried out using the regression facilities in GenStat (VSN International Ltd. 2011). Unbalanced ANOVA was used to generate predicted means and standard errors for the model term ‘variety’, referring to wheat variety, for each quality trait measured. Fixed model terms used in the treatment structure were variety and replicate; with no blocking terms.

Grain quality data for the Australian wheat varieties collected was spatially adjusted to take into consideration trial plot effects. Spatial model analysis was performed using an irregular grid, as not all of the wheat varieties in a trial were collected. The data for each quality trait, or variates, were analysed and a Y position and a X position was specified based on the row and column location of the wheat variety in the rectangular grid trial plot. Spatial model analysis, analysed two-dimensional data in the form of a grid using the method of residual or restricted maximum likelihood (REML) (VSN International Ltd. 2011). The analysis used ‘variety’, referring to wheat variety, as the fixed term and the model was fitted with power variance to rows and Euclidean covariance to columns. The irregular spatial model analysis generated adjusted means and standard errors for each quality trait, for each wheat variety, within a trial. In addition, residual plots for each quality trait, within a trial, were generated to assess the normal distribution and to check for outliers.

Correlations were performed to investigate relationships between the flour quality traits measured, between the chapatti quality traits measured, and between the flour quality traits and the chapatti quality traits measured. Significant correlations were identified using critical values for Pearson’s correlation coefficients at the 5% level of significance ($p<0.05$).

Principal component analysis (PCA) is a multivariate analysis technique used to find orthogonal linear combinations of a set of variables by transforming them into a set of linearly uncorrelated variables called principal components (VSN International Ltd. 2011). The analysis maximises the variation contained within the variables and, in doing so displays most of the original variability in a smaller number of dimensions; with the greatest variance accounted for in the first principal component (VSN International Ltd. 2011). PCA identified the important combinations of quality traits which were determining differences between the wheat varieties in the data sets analysed; and thus reduced the dimensionality of the data. The analysis operated using a correlation matrix to standardise the variates as they did not all share a common scale. Biplots of the PCA results were generated to provide graphic visualisation of the first two principal components (PC-1 and PC-2) driving variation within a data set. The variates were displayed as vectors and the wheat variety samples as points on the biplot.

Meta-analysis was used to generate GGE biplots for the grain quality data collected, for wheat varieties grown in five different environmental locations. A principal component analysis, on the variables, was performed to measure the genotype and genotype-by-environment variation (GGE). The environmental effects are removed from the analysis, as the effect of environment is usually the dominant source of variation. The meta-analysis instead concentrates on the genotype variation and genotype-by-environment interaction. The results obtained were used to calculate environment and genotype scores and these scores were then used to plot various attributes of the genotypes and environments (VSN International Ltd. 2011). The GGE biplots produced visually display the results and the performance of genotypes in different environments.

4.0 RESULTS AND DISCUSSION

4.1 Grain quality characterisation of Australian wheat varieties

4.1.1 Introduction

Grain quality is influenced by genotype, environmental factors, and their interactions. The growing location and environmental conditions during a season can therefore have significant influence on the quality attributes of wheat. Thus, grain quality, a consequence of these interactions, is assessed after each wheat harvest. The grain receival standards facilitate wheat grading and segregation into different grades based on grain quality and genotype. In Australia, each commercially released wheat variety has been classified into a particular grade; which includes APH, AH, APW, APWN, APDR, ASFT, ASWN and ASW. The grain however also needs to meet the receival standards of the designated grade to receive the appropriate payment. Grain which does not meet the quality criteria for a specific grade will be downgraded; and in exceptional circumstances if it does not meet the quality criteria for the specified human food milling grade it will be downgraded into one of the animal feed grades.

In this research, the Australian grain samples harvested and collected for chapatti making and evaluation were assessed and the quality of the wheat varieties in each of the trials was characterised. The Australian wheat varieties selected for study were grown in a number of locations around the state of W.A. These locations are termed National Variety Trials (NVT) and are referred to as ‘trial’ or ‘trials' within this thesis. A comparison between the trials, based on grain quality, was made to determine if the trials were significantly different to each other; and to assess which trials were suitable and had acceptable quality for further evaluation. The grain quality of the wheat varieties was also compared to Australian wheat receival standards to determine if the varieties met the specifications for their respective grade classification.

4.1.2 Materials and Methods

Summary of materials and methods

Twenty five commercially released Australian wheat varieties were evaluated from five different geographical locations. The selected wheat varieties were grown in NVT trials at the locations of Binnu, Eradu, Mukinbudin, Munglinup and Williams; which collectively represented a range of different growing environments found in W.A. It should be noted that one wheat variety, *Peake*, was not grown in the trials at Binnu and Eradu; and three wheat varieties, *Arrino*, *Yandanooka* and *Zippy*, were not grown in the trial at Munglinup. Each wheat variety was grown with three replications within a trial. For field trial plots, it is common practice to grow three replications of a wheat variety within a trial based on a randomised block design. Each of the replicates is grown in a different position and therefore in a specific location within the trial; and this minimises bias due to environmental influences. The replicates were labelled as, A, B and C, and the quality of each wheat variety replicate was individually assessed. A range of grain quality traits were measured and included; hectolitre weight, percentage of screenings, percentage of whole and broken grain, falling number, assessment of stained grain, and NIR predictions for wheat protein, flour yield, flour swelling volume, *CIE b**, particle size index and water absorption; as described in Table 3.2.

Statistical methods

Spatial model analysis was performed using an irregular grid to account for within trial environmental variation, such as soil variability, and thus consequently aimed to increase the precision of the data collected. The measurements taken for each of the grain quality traits and the within trial coordinates that the wheat variety replicates were grown at, within a trial, were used for spatial model analysis. The analysis used ‘variety’, referring to wheat variety, as the fixed term and the model was fitted with power variance structure to rows and Euclidean covariance to columns. The irregular spatial model analysis was repeated for each of the five trials and generated adjusted means and standard errors for each quality trait, for each wheat variety, within a trial. In addition, residual plots were generated to assess the normal distribution and check for outliers, for each quality trait within a trial. The spatially adjusted means were then used for subsequent statistical analyses.

Principal component analysis (PCA) was conducted using selected grain quality trait data of the wheat varieties in each of the five trials. Hectolitre weight, protein content, predicted yield and percentage of screenings were the grain quality traits used in the analysis; and are also referred to as variates. PCA used a correlation matrix to standardise the variates as they did not all share a common scale. A biplot of the PCA output was generated to visually display the first two principal components (PC-1 and PC-2) which were determining the greatest variation within a data set. The variates are displayed as vectors and the wheat varieties as points on the biplot. A convex hull was created for each trial, which clustered the wheat varieties within a trial.

Meta-analysis was performed to generate GGE (genotype and genotype-by-environment) biplots using the data of selected grain quality traits for the wheat varieties grown in each of the five trials. The meta-analysis used PCA and this was applied to each of the grain quality traits to display the genotype and genotype-by-environment variation (GGE). The GGE biplots visually described the performance of genotypes, or wheat varieties, in the five different growing environments (trials) for each of the selected grain quality traits; hectolitre weight, protein content, predicted yield and percentage of screenings.

4.1.3 Results and Discussion

Grain quality characterisation of wheat varieties in each trial

The grain quality of the wheat varieties in each trial was characterised to enable selection of suitable trials for chapatti making and quality evaluation. A range of grain quality traits were measured including; hectolitre weight, percentage of screenings, percentage of whole and broken grain, falling number, assessment of stained grain, and NIR predictions for wheat protein, flour yield, flour swelling volume, *CIE b**, particle size index and water absorption. The quality results for each wheat variety replicate, A, B and C, were then spatially adjusted to derive a predicted mean for each quality trait, for each wheat variety, in the Binnu, Eradu, Mukinbudin, Munglinup and Williams trials; as summarised in Appendix 1.15 to 1.19. The following characteristics; hectolitre weight, percentage of screenings, percentage of whole and broken grain, wheat protein content, falling number and predicted yield, were used to assess the quality of each trial. Tables 4.1 to 4.5 presents the data range minimum (Min.) to maximum (Max.) values, mean and standard deviation (SD) of the aforementioned traits for each trial.

Table 4.1. The range, mean and standard deviation of selected grain quality traits for wheat varieties in the Binnu trial.

Grain Quality Trait	Unit	Range (Min. – Max.)	Mean ± SD
Hectolitre weight	kg/ hL	77.6 – 81.9	80.0 ± 1.2
Screenings	%	2.0 – 11.6	5.9 ± 1.9
• Percentage whole grain	%	0.01 – 4.2	1.1 ± 1.0
• Percentage broken grain	%	1.6 – 10.1	4.8 ± 1.9
Falling number	seconds	428 – 489	459 ± 31
Predicted yield	tonnes/ ha	2.5 – 3.4	3.0 ± 0.2
NIR wheat protein	%	11.0 – 12.4	11.7 ± 0.4

Table 4.2. The range, mean and standard deviation of selected grain quality traits for wheat varieties in the Eradu trial.

Grain Quality Trait	Unit	Range (Min. – Max.)	Mean ± SD
Hectolitre weight	kg/ hL	75.2 – 82.2	79.9 ± 1.9
Screenings	%	0.6 – 6.4	2.8 ± 1.5
• Percentage whole grain	%	0.3 – 5.9	2.4 ± 1.5
• Percentage broken grain	%	0.06 – 1.0	0.4 ± 0.2
Falling number	seconds	393 – 431	410 ± 20
Predicted yield	tonnes/ ha	1.5 – 2.6	2.2 ± 0.2
NIR wheat protein	%	9.1 – 12.2	10.1 ± 0.6

Table 4.3. The range, mean and standard deviation of selected grain quality traits for wheat varieties in the Mukinbudin trial.

Grain Quality Trait	Unit	Range (Min. – Max.)	Mean ± SD
Hectolitre weight	kg/ hL	74.1 – 80.8	78.0 ± 1.5
Screenings	%	1.9 – 9.0	4.0 ± 1.6
• Percentage whole grain	%	1.2 – 8.2	3.1 ± 1.7
• Percentage broken grain	%	0.4 – 2.4	1.0 ± 0.6
Falling number	seconds	449 – 522	494 ± 40
Predicted yield	tonnes/ ha	1.1 – 1.8	1.5 ± 0.2
NIR wheat protein	%	10.8 – 13.2	11.7 ± 0.6

Table 4.4. The range, mean and standard deviation of selected grain quality traits for wheat varieties in the Munglinup trial.

Grain Quality Trait	Unit	Range (Min. – Max.)	Mean ± SD
Hectolitre weight	kg/ hL	75.3 – 81.6	77.2 ± 1.5
Screenings	%	1.0 – 5.3	2.5 ± 1.1
• Percentage whole grain	%	0.3 – 4.9	1.6 ± 1.2
• Percentage broken grain	%	0.3 – 2.0	0.9 ± 0.4
Falling number	seconds	387 - 457	416 ± 36
Predicted yield	tonnes/ ha	3.1 – 4.3	3.8 ± 0.3
NIR wheat protein	%	10.0 – 11.8	11.1 ± 0.5

Table 4.5. The range, mean and standard deviation of selected grain quality traits for wheat varieties in the Williams trial.

Grain Quality Trait	Unit	Range (Min. – Max.)	Mean ± SD
Hectolitre weight	kg/ hL	80.6 – 85.5	82.7 ± 1.2
Screenings	%	2.5 – 10.1	6.5 ± 2.0
• Percentage whole grain	%	1.0 – 7.9	3.0 ± 1.9
• Percentage broken grain	%	0.6 – 7.5	3.4 ± 1.5
Falling number	seconds	327 – 436	398 ± 61
Predicted yield	tonnes/ ha	2.0 – 4.4	3.8 ± 0.5
NIR wheat protein	%	9.1 – 12.9	10.3 ± 0.7

The quality data of the wheat varieties assessed was compared to Australian grain receival standards; *Wheat Standards, Grain Trade Australia* (Grain Trade Australia 2010). The Australian wheat varieties studied were classified into the following grades; Australian Hard (AH), Australian Premium White (APW), Australian Standard White (ASW), Australian Standard White Noodle (ASWN) and Feed, for one wheat variety *Kennedy*. The wheat receival standards for these grades were used to guide the quality evaluation, for the respective wheat variety.

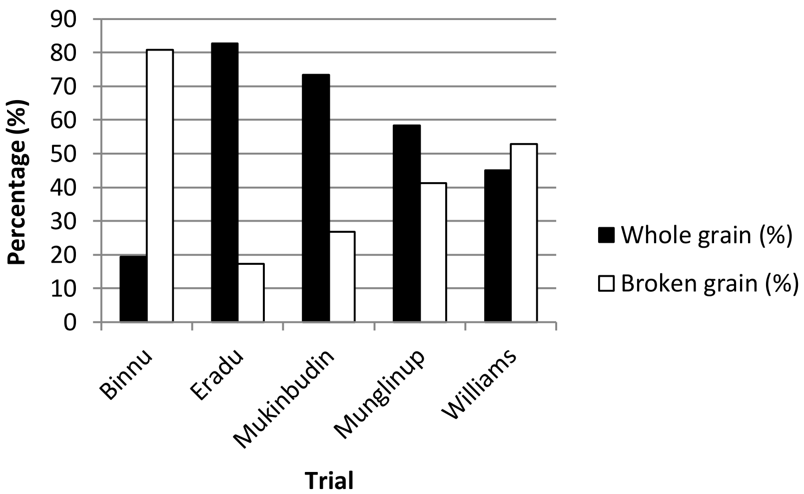
Hectolitre weight, a bulk density measurement, provides information about how the grain fills during the final stages of growth; especially the amount of starch that is laid down in addition to the protein. Low hectolitre weight values can indicate under filled grain which has implications on milling yield and processing ability because of the reduced amount of starch and protein available (AWB Limited n.d.). The minimum hectolitre weight requirement across all assessed grades was 74.0 kg/ hL, except for the Feed grade which was 62.0 kg/ hL, in 2010 (Grain Trade Australia 2010). The grain samples of each wheat variety collected for the research met these requirements with all of the hectolitre weight measurements greater than 74.0 kg/ hL. On average the wheat varieties in the Williams trial had the highest hectolitre weight, 82.7 ± 1.2 kg/ hL; and the wheat varieties in the Munglinup trial had the lowest mean hectolitre weight, 77.2 ± 1.5 kg/ hL. The hectolitre weight values obtained were comparable to NVT trial data for the 2009 main wheat season in W.A.; and the NVT hectolitre weight results were also shown to vary at different trial locations (National Variety Trials and Australian Crop Accreditation System Limited 2010). It was therefore concluded that the wheat varieties met minimum standards for hectolitre weight and had acceptable quality, in regards to grain filling with well filled not shrivelled grain.

Screenings are an important indicator of grain quality and for the wheat varieties assessed, a maximum of 5.0% screenings were allowed for each of the grades of interest, and a maximum of 15.0% screenings for the Feed grade (Grain Trade Australia 2010). It was identified that each trial contained wheat varieties which exceeded the 5.0% screenings maximum. The Williams trial had the highest mean percentage of screenings, 6.5 ± 2.0%, followed by the Binnu trial with 5.9 ± 1.9%.

The trial which had the lowest mean percentage of screenings was the Munglinup trial with 2.5 ± 1.1%. Although all trials met hectolitre weight requirements, the high percentage of screenings can indicate shrivelled and under filled grain; and inadequate grain filling is primarily an effect of the growing environment (Blum 1998). For example, in W.A. dry spring conditions have been shown to result in high screenings (Shackley et al. 2011).

Further assessment of the screenings, characterised the percentage of whole and broken grains in the screenings recovered for each wheat variety. Figure 4.1 shows the mean percentage of whole and broken grains for each trial. Whole or intact grains present in the screenings, generally represented shrivelled or under filled grain; and the broken grains recovered can be a result of harvesting. In the Binnu trial, 17 of 24 wheat varieties assessed had equal to, or greater than 80% of broken grains in the screenings evaluated. Conversely, for 21 of 24 wheat varieties in the Eradu trial, the screenings contained 75% or greater whole grains. The screenings of the wheat varieties in the Mukinbudin trial were also similar to the Eradu trial, with a higher mean percentage of whole grains, 73%, than broken grains. For the Munglinup and Williams trials the mean percentage of whole grain and broken grains were shown to be similar; see Figure 4.1. Nonetheless, although the screenings indicated some shrivelled grain, in all trials this matter was removed so only sound well filled grain was used in subsequent research.

Figure 4.1. Mean percentage of whole grain and broken grain in the screenings of wheat varieties in each of the five trials.



The falling number test was used to assess α-amylase activity and selected wheat varieties *Tammarin Rock*, *Westonia* and *Wyalkatchem* were used as the indicator varieties for each trial. An indicator variety in this instance referred to wheat varieties which were known to be more susceptible to pre-harvest sprouting, which results in increased α-amylase activity and lower falling numbers (Young and Ellis 2012). If the wheat varieties, which were more susceptible to pre-harvest sprouting, had acceptable falling numbers, then it was expected that the less susceptible wheat varieties would also have acceptable falling numbers. A minimum falling number of 300 seconds was the grade standard for these three wheat varieties. It was determined that *Tammarin Rock*, *Westonia* and *Wyalkatchem* all had falling numbers of greater than 300 seconds. Thus they met grade specifications for falling number, and indicated that the grain was of sound quality with minimal enzyme activity. It is important that grain meets minimum falling number requirements as grain storage, processing and end product quality can be affected from increased α-amylase activity due to the changes it makes to the starch component of the endosperm (Mares and Mrva 2008).

Predicted yields were obtained from NVT trial data as the exact yields were not able to be determined; as after the grain was harvested, it was divided and samples were taken for NVT testing and the remainder of the grain was collected for this research. (National Variety Trials and Australian Crop Accreditation System Limited 2009). The wheat collected however was weighed and the grain weights were found to reflect the NVT wheat variety predicted yields (National Variety Trials and Australian Crop Accreditation System Limited 2010). The wheat varieties in the Munglinup and the Williams trials had the highest mean yield, 3.8 tonnes/ ha, followed by the Binnu trial, the Eradu trial, and lastly the Mukinbudin trial which had the lowest mean yield of 1.5 tonnes/ ha. Across all of the trials, the wheat variety yields ranged from 1.1 to 4.4 tonnes/ ha. It was important for this research, that sufficient grain of each wheat variety in a trial was available for further testing and research.

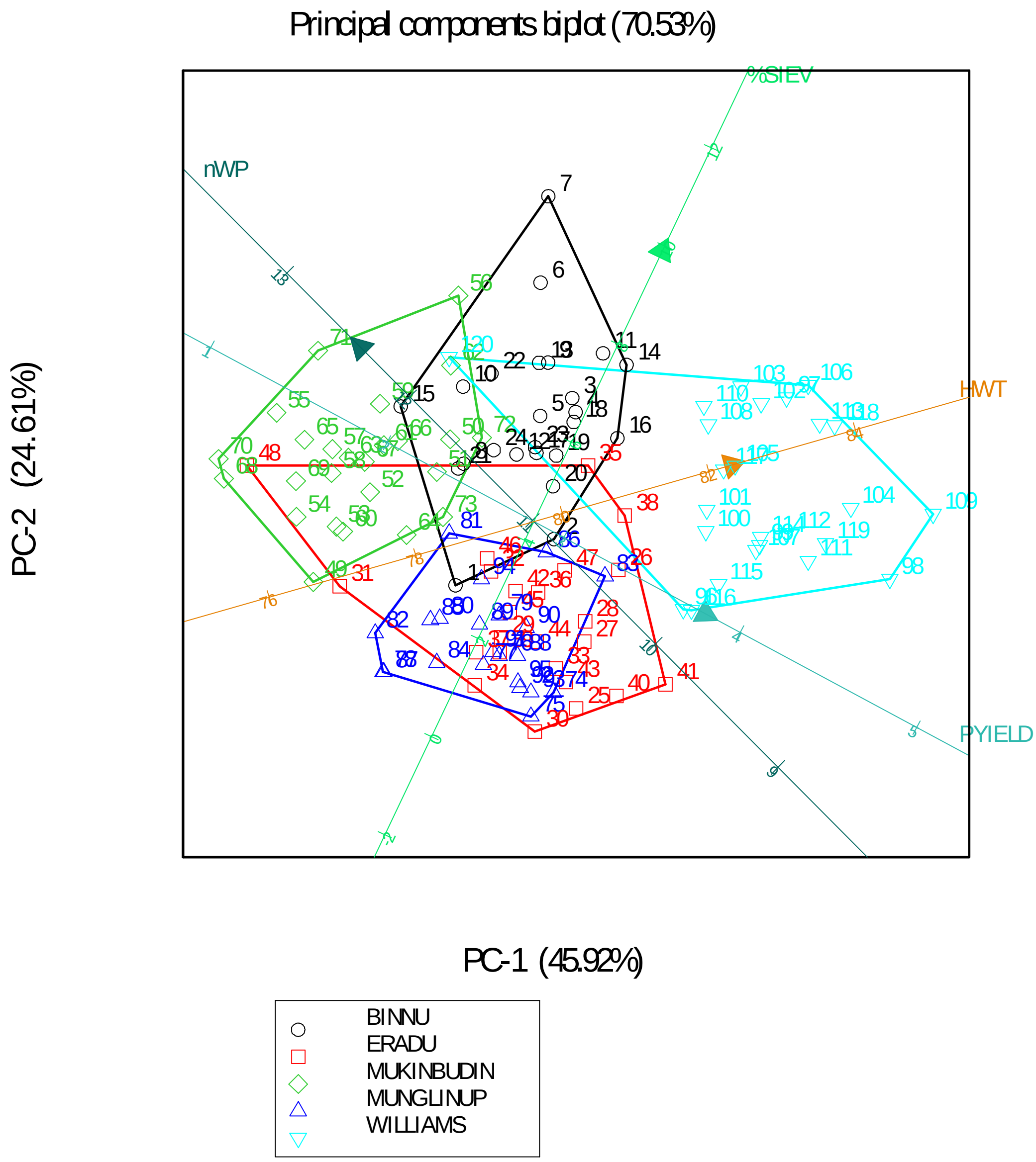
The wheat protein content was assessed by NIR and in this section *4.1 Grain quality characterisation of Australian wheat varieties* where 'protein content' is stated, it does refer to NIR predicted protein content. The protein content was found to vary across the trials; the mean protein content was 10.1% and 10.3% for the Eradu and the Williams trials; respectively. The Munglinup trial had a mean protein content of 11.1%, and the Binnu and the Mukinbudin trials both had a mean protein content of 11.7%. Across all of the trials, the protein content of the wheat varieties was determined to range from 9.1 to 13.2%, and so met the protein content requirements reported to be suitable for chapatti making, which ranges from 10 to 12% (Gupta 2004; Nagarajan 2004a). The wheat samples collected for chapatti research largely met these requirements.

Stained grain assessment was also conducted to further evaluate the grain quality of wheat varieties in the Binnu and the Williams trials. All of the wheat varieties in the Binnu trial, except for one *Guardian*, contained stained grains in one or more of the replicates collected. The Williams trial, in comparison, had a lower number of wheat varieties with stained grains, and the percentages of stained grain were also lower than in the Binnu trial. Nonetheless, the percentages of stained grain in both trials were below the grade standard maximum limits of 5.0% for stained grains and 2.0% for pink stained grain; so the wheat varieties in both trials met the grade standard. Low percentages of stained grains are required, as stained grains typically result from fungal diseases such as Fusarium head blight (Loughman, Thomas, and Wright 2004). Infection of crops by *Fusarium* spp. affects crop and grain quality, but is also an issue due to the mycotoxins which can be produced, and which are harmful to animals and humans in significant quantities (Loughman, Thomas, and Wright 2004).

Determination of differences in grain quality between trials

It was important to understand the similarities and differences in grain quality between the trials, as not all five trials were able to be studied for chapatti making and quality evaluation. Principle component analysis (PCA) was performed to compare the trials using the grain quality traits of hectolitre weight, predicted yield, protein content and percentage of screenings. A biplot of the PCA results is shown in Figure 4.2 and the PCA output is described in Appendix 1.20. The wheat varieties in a trial are surrounded by a coloured convex hull which represents a trial. The Binnu trial is positioned in the centre at the top of the biplot and is black. The Eradu trial is in the centre of Figure 4.2 at the bottom in red. The Mukinbudin trial is green and on the left hand side of the biplot. The Munglinup trial is dark blue overlapped with the Eradu trial in the centre at the bottom; and the Williams trial is on the right hand side of the biplot in light blue.

Figure 4.2. Biplot of PCA of selected grain quality traits for the Australian wheat varieties in five trials.

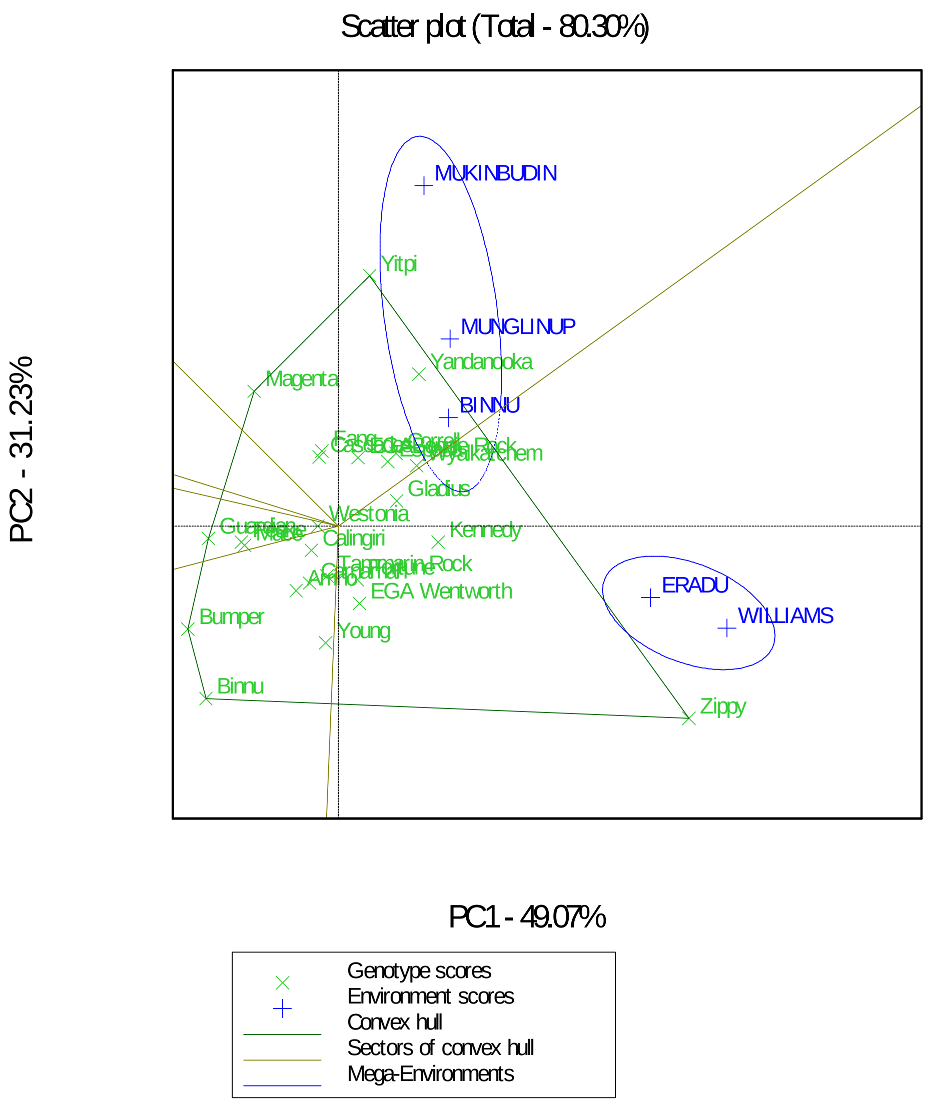


The first principle component (PC-1) accounted for 45.92% of the variability between the trials, and was driven by the three traits; hectolitre weight, protein content and predicted yield. Protein content was observed to be negatively correlated to hectolitre weight, predicted yield and percentage of screenings. Figure 4.2 demonstrates this as hectolitre weight and predicted yield increase in value horizontally from left to right of the biplot. Whereas, protein content is shown to increase horizontally from right to left of the biplot. In addition, the percentage of screenings is seen to increase vertically from the bottom to the top of the biplot; and is a main contributing factor to the second principal component (PC-2) which was determining 24.61% of the differences in grain quality between the trials.

Ideally, the trials selected for chapatti making and quality evaluation should contain wheat varieties with high hectolitre weights, high predicted yields and low screenings. The wheat varieties in the Eradu, the Munglinup and the Williams trials largely have these attributes as shown in Figure 4.2. Higher hectolitre weights and predicted yields are to the right hand side of the biplot, and lower percentage of screenings towards the bottom of the biplot. The Eradu and the Munglinup trials were identified as having similar grain quality, illustrated by the overlap of the trials on the biplot. It was also observed that the grain quality of the Binnu trial was similar to the Eradu and the Munglinup trials, but its higher position on the biplot indicated higher percentage of screenings. Moreover, the wheat varieties in the Mukinbudin trial were shown to have lower hectolitre weight and predicted yield values, in comparison to the other trials. Overall, each of the trials was determined to have different grain quality, except for the Eradu and the Munglinup trials which had some similarities.

In regards to selecting trials for chapatti quality evaluation, protein content was an important consideration and trials with different protein content but within the range suitable for chapatti making of 10 to 12% were required. GGE biplots were generated for each of the selected grain quality attributes, to further describe the differences between trials for specific quality traits. Figure 4.3 is an example of the GGE biplots generated and depicts the quality trait of NIR predicted protein content. The GGE biplot, Figure 4.3, shows grouping of the Binnu, the Munglinup and the Mukinbudin trials as having similar protein content. The Eradu and the Williams trials are also clustered together, indicating that they also have comparable protein content. The mean protein content of the wheat varieties in the Binnu, the Munglinup and the Mukinbudin trials ranged from 11.0 to 11.7%. Whereas, the mean protein content of the Eradu and the Williams trials ranged from 10.1 to 10.3%. Figure 4.2 also confirmed these differences in protein content by the position of the trials along the vector for protein content. The GGE biplots generated for the grain quality traits of hectolitre weight, predicted yield and percentage of screenings are displayed in Appendix 1.21. Therefore, when taking into consideration the differences in protein content between the trials; the Binnu or the Munglinup trial, and the Williams or the Eradu trial, should be selected for the research. Previously the trials of Eradu and Munglinup were identified as having similar grain quality, see Figure 4.2; therefore the Binnu and the Williams trials would be suitable for chapatti research.

Figure 4.3. GGE biplot for the grain quality trait of NIR predicted protein content.



Grading and classification of Australian wheat varieties

Each commercially released Australian wheat variety is classified into a particular grade based on processing and end product quality attributes. Thus each wheat variety has the potential to meet the requirements of a specified grade after harvest. It is important for grain to obtain the designated grade primarily due to the economic implications; however it also signifies that the grain has met standard quality requirements and has the potential to make quality end products. The grain quality data collected for hectolitre weight, protein content, percentage of screenings and falling number, was used to classify the wheat varieties collected according to the bin grade cascade; as outlined in *Wheat Standards, Grain Trade Australia* (Grain Trade Australia 2010). Table 4.6 tabulates each wheat variety's ability to meet the quality standards of their respective grade. Appendix 1.22 summarises the specified and the actual grade that each of the wheat varieties met.

Table 4.6. Ability of Australian wheat varieties to meet grade requirements.

LEGEND: + = met grade requirements (white) | 2 = meet grade 2 requirements (blue)
- = did not meet grade requirements (green)

Australian Hard

Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)	Falling number (seconds)
Minimum 74.0	Maximum 5.0	Minimum 13.0 (Grade 1); Minimum 11.5 (Grade 2)	Minimum 300

Wheat Variety	Trial	Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)
Carnamah	Binnu	+	-	2
	Eradu	+	+	-
	Mukinbudin	+	+	-
	Munglinup	+	+	-
	Williams	+	-	-
Cascades	Binnu	+	-	2
	Eradu	+	+	-
	Mukinbudin	+	+	2

	Munglinup	+	+	-
	Williams	+	-	-
EGABonnie Rock	Binnu	+	-	2
	Eradu	+	+	-
	Mukinbudin	+	+	2
	Munglinup	+	+	2
	Williams	+	-	-
Mace	Binnu	+	-	-
	Eradu	+	+	-
	Mukinbudin	+	+	-
	Munglinup	+	+	-
	Williams	+	-	-
Tammarin Rock	Binnu	+	-	-
	Eradu	+	+	-
	Mukinbudin	+	+	2
	Munglinup	+	+	-
	Williams	+	-	-
Yitpi	Binnu	+	-	2
	Eradu	+	+	-
	Mukinbudin	+	+	+
	Munglinup	+	+	2
	Williams	+	-	-

Australian Premium White

Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)	Falling number (seconds)
Minimum 74.0	Maximum 5.0	Minimum 10.5 (Grade 1); Minimum 10.0 (Grade 2)	Minimum 300

Wheat Variety	Trial	Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)
Correll	Binnu	+	-	+
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	2

EGA Wentworth	Binnu	+	+	+
	Eradu	+	+	+
	Mukinbudin	+	-	+
	Munglinup	+	+	+
	Williams	+	-	2
Espada	Binnu	+	-	+
	Eradu	+	+	-
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	+
Fang	Binnu	+	-	+
	Eradu	+	-	2
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	-
Gladius	Binnu	+	-	+
	Eradu	+	+	2
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	+
Magenta	Binnu	+	-	+
	Eradu	+	+	-
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	-
Peake	Binnu	N/A	N/A	N/A
	Eradu	N/A	N/A	N/A
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	-	-
Westonia	Binnu	+	-	+
	Eradu	+	+	-
	Mukinbudin	+	+	+
	Munglinup	+	+	2
	Williams	+	+	2
Wyalkatchem	Binnu	+	+	+

	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	+	+
Young	Binnu	+	-	+
	Eradu	+	+	2
	Mukinbudin	+	-	+
	Munglinup	+	+	+
	Williams	+	-	2
Zippy	Binnu	+	-	+
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	N/A	N/A	N/A
	Williams	+	+	+

Australian Standard White

Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)	Falling number (seconds)
Minimum 74.0	Maximum 5.0	No minimum	Minimum 300

Wheat Variety	Trial	Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)
Bumper	Binnu	+	-	+
	Eradu	+	+	+
	Mukinbudin	+	-	+
	Munglinup	+	+	+
	Williams	+	-	+
Guardian	Binnu	+	-	+
	Eradu	+	-	+
	Mukinbudin	+	-	+
	Munglinup	+	-	+
	Williams	+	-	+

Australian Standard White Noodle

Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)	Falling number (seconds)
Minimum 74.0	Maximum 5.0 (Grade 1); Maximum 10.0 (Grade 2)	Minimum 9.5 to Maximum 11.5 (Grade 1); No minimum or maximum (Grade 2)	Minimum 300

Wheat Variety	Trial	Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)
Arrino	Binnu	+	+	+
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	N/A	N/A	N/A
	Williams	+	+	+
Binnu	Binnu	+	+	+
	Eradu	+	2	2
	Mukinbudin	+	2	+
	Munglinup	+	+	+
	Williams	+	2	+
Calingiri	Binnu	+	2	2
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	2	+
Fortune	Binnu	+	+	2
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	2	+
Yandanooka	Binnu	+	+	2
	Eradu	+	+	+
	Mukinbudin	+	+	2
	Munglinup	N/A	N/A	N/A
	Williams	+	2	+

Feed

Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)	Falling number (seconds)
Minimum 62.0	Maximum 15.0	No minimum or maximum	Not applicable

Wheat Variety	Trial	Hectolitre weight (kg/ hL)	Screenings (%)	Protein (%)
Kennedy	Binnu	+	+	+
	Eradu	+	+	+
	Mukinbudin	+	+	+
	Munglinup	+	+	+
	Williams	+	+	+

As previously reported, hectolitre weight requirements were met for all wheat varieties and therefore all wheat varieties met this grade requirement. Furthermore, based on the falling number results, for the three indicator wheat varieties tested, it was anticipated that all wheat varieties would therefore meet the minimum falling number requirements for each grade. Consequently, protein content and percentage of screenings were the determining factors used to grade the wheat varieties collected. Table 4.7 shows the percentage of wheat varieties in each trial which met their respective grade requirements. The Binnu trial had the lowest percentage, 33% of wheat varieties, which met grade specifications, and the Williams trial was also similar with 36% of the wheat varieties in the trial meeting grade requirements. Conversely, the Munglinup trial had the highest percentage, 77% of wheat varieties which met designated grade requirements, of the five trials assessed.

Table 4.7. Percentage of wheat varieties which met their respective grade requirements in each trial.

Trial	Number of wheat varieties evaluated in the trial	Percentage of wheat varieties which met their respective grade requirements (%)
Binnu	24	33
Eradu	24	54
Mukinbudin	25	76
Munglinup	22	77
Williams	25	36

Protein content is a key quality attribute and is used to distinguish the different classes of wheat due to its influence on processing and end product quality. It was identified that there were no wheat varieties of the AH grade, except for *Yitpi* from the Mukinbudin trial, that met the minimum 13.0% protein content requirements for grade one. Four wheat varieties, *Carnamah*, *Cascades*, *EGA Bonnie Rock*, and *Yitpi*, from the Binnu trial, however met grade two AH requirements of a minimum 11.5% protein content. In addition, two wheat varieties from the Mukinbudin and the Munglinup trials met grade two AH criteria; but there were no wheat varieties from the Williams trial which met AH grade one or two protein content requirements. The wheat varieties classified as belonging to the APW grade, met grade one or grade two APW protein content specifications in all trials; except for three wheat varieties. It was also observed that the wheat varieties from the Eradu and the Williams trials were mainly classified as APW grade two; having protein contents between 10.0 to 10.5%. The ASWN/ ANW grade has a minimum and maximum protein content range of 9.5 to 11.5% for grade one and no set limits for grade two. Therefore all wheat varieties from the ASWN grade met grade one or two requirements for protein content. It was noted however, that the majority of the wheat varieties met grade one ASWN specifications. The ASW and Feed grades had no set minimum or maximum protein content requirements; thus the three wheat varieties belonging to these two grades met the protein content criteria.

The percentage of screenings was the second quality trait which determined the ability of wheat varieties to meet grade receival specifications. It was found that wheat varieties from the Binnu and the Williams trials, predominantly produced grain which exceeded the maximum percentage screening requirements for their respective grades. 67% of the wheat varieties from the Binnu trial, and 64% of the wheat varieties from the Williams trial had greater than the maximum allowable screenings. The receival standard limit, for the AH, APW and ASW grades, was a maximum of 5% screenings (Grain Trade Australia 2010). Furthermore, it was determined that the AH wheat varieties, which had screenings greater than 5%, were from the Binnu and the Williams trials. For the APW wheat varieties, only *Wyalkatchem* had screenings of less than 5% for all of the trials. The two wheat varieties, *Bumper* and *Guardian*, of ASW grade had greater than 5% screenings for all trials, except the wheat samples of *Bumper* which were from the Eradu and the Munglinup trials. In addition, all of the ASWN grade wheat varieties met the screening requirements for grade one, maximum 5.0% screenings; or grade two, maximum 10.0% screenings (Grain Trade Australia 2010). Lastly, wheat variety *Kennedy* met the Feed grade requirements for all grain samples with screenings less than 15%.

4.1.4 Conclusion

The grain quality of the Australian wheat varieties, collected from five different geographically located trials, was characterised to determine their suitability for chapatti making and quality evaluation. Overall the wheat varieties were of acceptable quality, and no grain samples were downgraded from a food grade to a feed grade. All of the wheat varieties met hectolitre weight requirements which indicated adequate grain filling; and were predicted to meet falling number requirements, indicating sound grain, based on the results of selected wheat varieties. The protein content although variable, both between and within the trials, largely reflected the protein content range of 10 to 12%, which is suitable for chapatti making. Two trials, the Binnu and the Williams trials, were selected for further study. These two trials were chosen as they had significant differences in protein content, a mean protein content of 11.7% and 10.3%, respectively. Although the percentage of screenings were higher in these two trials, the wheat samples were physically screened to remove matter less than 2.0 mm, and only sound well filled quality grains were used in the subsequent research. Also as indicated in the Binnu trial, the screenings were mainly found to be broken grain, a result of mechanical harvesting, and not shrivelled, under filled grains. The percentage of stained grains was also acceptable for wheat varieties in these two trials.

4.2 Physicochemical and rheological characterisation of chapatti flours and their relationship with chapatti quality traits

4.2.1 Introduction

Wheat quality can generally be understood as the ability of wheat to make quality end product; with different products having different quality requirements. Furthermore, wheat quality can be defined by characterising physical, chemical and rheological properties, and ideally test baking of the particular end product. There are few studies which have systematically investigated interactions between wheat properties and their relationship to chapatti quality. The studies which have been conducted, have predominantly yielded knowledge of wheat quality requirements for chapatti made from Indian wheat varieties (Chavan, Gaikwad, and Chavan 2007; Sharma et al. 2004; Prabhasankar, Manohar, and Gowda 2002; Rao and Bharati 1996; Choubey, Nanda, and Gautam 1987; Shurpalekar et al. 1976) and the chapatti making ability of Australian wheat has not been investigated.

Chapatti is flat unleavened bread and traditionally made from stone milled whole wheat flour, or *atta* as it is referred to in India. The atta is mixed with water to form dough, rested and sheeted into a circle of approximately 150 mm in diameter and 2 mm thick. The sheeted dough is baked on both sides, on a cast iron griddle pan, and the chapatti temporarily steam leavened to create two layers.

Whole wheat chapatti is ideally creamy in colour with a scattering of brown spots over the surface (Hemalatha et al. 2007). Full puffing of chapatti is also desired so that two distinct layers are formed (Ghodke, Ananthanarayan, and Rodrigues 2009). The texture of chapatti should be soft and pliable, not leathery or brittle (Srivastava, Prasada Rao, and Haridas Rao 2003). The ability of the chapatti to tear and fold easily are important quality traits. In addition, chapatti should have a slight soft chewy texture in the mouth, a sweetish taste and pleasant wheaty aroma (Ghodke and Ananthanarayan 2007; Ur-Rehman, Paterson, and Piggott 2007a; Gujral and Pathak 2002). Wheat quality requirements reported for chapatti include a protein content of 10 to 12%, protein of medium dough strength and properties such as high damaged starch and water absorption contribute towards good quality chapatti (Nagarajan 2006).

The suitability of Australian wheat for production of the traditional and staple Indian food, chapatti, was investigated in this research. The first objective was to characterise and compare the physicochemical and rheological quality of the Australian and the Indian chapatti flours. The second objective was to characterise and compare the quality of chapattis made from the Australian and the Indian chapatti flours. Lastly, further understanding of the wheat quality requirements for chapatti was determined by examining relationships between wheat flour properties and chapatti quality traits.

4.2.2 Materials and Methods

Summary of materials and methods

Australian wheat varieties grown at two different locations, Binnu and Williams in W.A., were harvested and collected from the 2009 season. Twenty four and twenty five commercially released Australian wheat varieties were chosen for study from these two trials, Binnu and Williams; respectively. Six Indian wheat varieties were obtained, as flour, from India and were grown in the states of Haryana and Punjab in the 2008/ 09 season. The Indian wheat varieties varied in their chapatti making abilities and were used as benchmarks for the Australian wheat varieties. Furthermore, Indian wheat varieties *HI 1531* and *PBW 175* were used as the 'gold' standards in this study. These wheat varieties have been reported as having good chapatti quality and they also demonstrated these qualities in this research. The grain samples were milled on a laboratory stone mill to produce whole wheat flour; and standard wheat quality tests were conducted to characterise flour quality. (Refer to section *3.4 Wheat and flour quality characterisation* for further details). Chapattis, 150 mm in diameter and 2 mm thick were prepared using a standard method. Chapatti quality attributes were evaluated and included; analysis of chapatti texture using extensibility tests on the texture analyser (Stable Microsystems); measurement of chapatti colour using a Minolta Chroma Meter (Konica Minolta); and measurement of puffed height and bake loss was also conducted.

Statistical methods

The flour and chapatti quality trait data collected was grouped into data sets related to genotype and the environment the samples were grown in. The data was initially grouped as a Binnu data set, a Williams data set and an Indian data set. The preliminary data analysis conducted however, identified an effect of grain hardness on the statistical output. Therefore the data was further categorised into the following groups; Binnu Hard, Binnu Soft, Williams Hard, Williams Soft and Indian; referring to the location the wheat varieties were grown, and the hard and soft wheat varieties in each data set. The Indian wheat varieties, *C 306*, *HI 1531*, *K 9107*, *PBW 175*, *PBW 343* and *WH 542*, were all classified as hard wheats.

The predicted means and standard errors were determined for each flour quality and chapatti quality trait, for each wheat variety, by running unbalanced ANOVA on the data collected. The unbalanced ANOVA used the flour or chapatti quality traits as the Y-variate and the treatment as 'variety', referring to wheat variety; there were no blocking terms. The data sets were unbalanced due to variation in the number of wheat varieties in each data set and from variation in the number of replicate measurements performed for the different quality tests. For example, the ash content for all wheat varieties was measured in triplicate; however measurement of the chapatti colour traits resulted in 54 measurements per wheat variety.

Correlations were performed to identify significant correlations between flour quality traits; between chapatti quality traits; and significant relationships between flour and chapatti quality traits. Significant correlations were identified using critical values for Pearson's correlation coefficients at the 5% level of significance ($p<0.05$).

Principle component analysis (PCA) was performed to identify important combinations of quality traits which were determining differences between wheat varieties in the data sets analysed. The flour quality traits and the chapatti quality traits were analysed separately for each data set, and four principal components were generated. PCA used a correlation matrix analysis to standardise the variates as they did not all share a common scale. Biplots were produced which visually displayed the first two principal components, PC-1 and PC-2, which were describing variation within a data set; and to compare similarities and differences between the data sets analysed.

4.2.3 Results and Discussion

Characterisation of the quality of Indian chapatti flours

A range of physicochemical and rheological properties were characterised to define the quality of the Indian chapatti flours. Predicted means of selected flour quality traits for the Indian wheat varieties are shown in Table 4.8. A table of predicted means which describes all of the flour quality traits measured for the Indian chapatti flours is presented in Appendix 1.23. In addition, the reported chapatti quality of the six Indian wheat varieties has been included in Table 4.8 (Coventry et al. 2011; Gujral 2009; Srivastava, Prasada Rao, and Haridas Rao 2003). It should also be noted that although grain of the Indian wheat varieties was not able to be obtained for analysis, hectolitre weight was assessed in India prior to milling. The hectolitre weight of the Indian wheat varieties ranged from 80 to 87 kg/ hL and indicated plump well filled grain, suitable for quality evaluation and processing.

Table 4.8. Predicted means of selected flour quality traits for the Indian wheat varieties.

Wheat Variety	Chapatti Quality	FN (sec)	Ash (%)	WP (%)	WA (%)	DS (%)	Ext (mm)	PSI0.9 (µm)
HI 1531	Good	677	1.30	11.8	86.0	14.5	85	323.0
PBW 175	Good	610	1.58	11.0	82.9	16.0	78	333.4
C 306	Good	493	1.64	10.4	77.5	14.4	54	350.4
K 9107	Good	626	1.72	11.4	74.7	12.2	90	320.0
PBW 343	Average	416	1.47	10.7	70.5	9.5	82	324.6
WH 542	Poor	541	1.32	11.4	74.4	11.9	76	382.7

LEGEND - **Ash**: ash content; **DS**: damaged starch content; **Ext**: dough extension; **FN**: falling number; **PSI0.9**: 90^{th} percentile of the particle size distribution; **WA**: farinograph water absorption; **WP**: protein content

The falling number for each of the Indian wheat varieties was determined to be greater than 300 seconds and indicated that the grain was not rain affected, had sprout damage or high levels of α-amylase activity (Mares and Mrva 2008;

Humphreys and Noll 2002). The ash content was observed to be high, ranging from 1.32 to 1.72%, but typical of Indian whole wheat flours of high extraction (Prabhasankar, Manohar, and Gowda 2002; Shurpalekar et al. 1976). The protein content ranged from 10.4 to 11.8% and this was within the range of 10 to 12% described to be suitable for chapatti making (Gupta 2004; Nagarajan 2004a).

The water absorption of the Indian chapatti flours was shown to range from 70.5 to 86.0% and the mean water absorption was 77.7 ± 5.8%. Comparable values for water absorption, 70 to 82.5%, have been reported for Indian wheat varieties milled using a stone mill or similar milling process (Hemalatha et al. 2007; Prabhasankar, Manohar, and Gowda 2002; Haridas Rao, Leelavathi, and Shurpalekar 1989). In previous studies, the chapatti flour water absorption and the damaged starch content have been observed to be significantly correlated ($p<0.001$); and to have a significant influence on chapatti quality ($p<0.01$) (Ghodke, Ananthanarayan, and Rodrigues 2009; Prabhasankar, Manohar, and Gowda 2002; Haridas Rao, Leelavathi, and Shurpalekar 1989). In this study, the quality traits of water absorption and damaged starch content were also found to be significantly and positively correlated ($p<0.01$).

The damaged starch content of the Indian chapatti flours ranged from 9.5 to 16.0% and were observed to be consistent with earlier reports of the damaged starch content of Indian chapatti flours (Hemalatha et al. 2007; Srivastava, Prasada Rao, and Haridas Rao 2003). Prabhasankar and Manohar (2002) however reported damaged starch contents of up to 24% for some Indian wheat varieties when using typical plate milling conditions. Nevertheless, Haridas Rao and Manohar (2003) stated a damaged starch content of 16 to 18% was important for chapatti quality; and Hemalatha et al. (2007) reported a damaged starch content of 14 to 16.5% made good quality chapatti. Softer texture chapatti, which is desired, results from flour with higher water absorption, and higher damaged starch is an important contributing variable to water absorption (Haridas Rao and Manohar 2003).

The quality trait of dough extension, measured by the Brabender extensigraph, ranged from 54 to 90 mm. The results determined were not unlike former published findings but were towards the lower end of the ranges reported. Extensibility has been reported to range from 40 to 58 mm (Haridas Rao, Leelavathi, and Shurpalekar 1989); 74.5 to 171.5 mm (Shurpalekar et al. 1976); and 96 to 127 mm (Hemalatha et al. 2007) for Indian chapatti flours. Few studies on Indian chapatti flour however, have included extensigraph testing as part of their assessment of protein and dough quality. Alternatively, the SDS sedimentation and farinograph tests have been prevalently used; and medium strong dough has generally been described as being suitable for chapatti making (Ur-Rehman, Paterson, and Piggott 2007a; Prabhasankar, Manohar, and Gowda 2002).

The final quality trait presented in Table 4.8, describes the particle size of the 90th percentile of the particle size distribution of a flour sample analysed. The results show that the particle size indexes, of the Indian wheat varieties, were comparable; with a difference of 62.7 μm between the highest and lowest result. Grain hardness can be assessed by measuring the particle size index of flour. Thus it may be concluded that the Indian wheat varieties studied, had similar grain hardness to each other (Pasha, Anjum, and Morris 2010). In addition, the milling process is known to influence the particle size of the resultant flour and a range of different milling procedures have been used to make Indian chapatti flours; as reported in the literature (Gill, Sodhi, and Kaur 2005; Prabhasankar and Haridas Rao 2001; Haridas Rao, Leelavathi, and Shurpalekar 1989; Sidhu et al. 1988; Orth 1977). A comparison of particle size index values is therefore challenging. Nevertheless, finer granulation flour has been described to be important for chapatti quality; and Indian medium hard grain has been defined as a wheat quality requirement for chapatti (Nagarajan 2006; Haridas Rao and Manohar 2003).

Collectively, for the Indian wheat varieties studied, the results obtained for the flour quality traits discussed correspond with previously reported characterisations of Indian chapatti flour quality.

Characterisation of the quality of Australian chapatti flours

The flour quality of the Australian wheat varieties collected from the Binnu and the Williams trials were characterised using the same physicochemical and rheological tests performed on the Indian chapatti flours. The predicted means for selected flour quality traits of selected Australian wheat varieties are described in Tables 4.9 and 4.10, for the Binnu and the Williams trials; respectively. Australian wheat varieties, representative of each grade evaluated, have been included in each table. The complete list of the Australian wheat varieties studied and the flour quality traits measured can be referred to in Appendix 1.24 and 1.25.

Table 4.9. Predicted means of selected flour quality traits for selected Australian wheat varieties from the Binnu trial.

Wheat Variety	Grade	FN (sec)	Ash (%)	WP (%)	WA (%)	DS (%)	Ext (mm)	PSI0.9 (µm)
HI 1531	*Indian*	*677*	*1.30*	*11.8*	*86.0*	*14.5*	*85*	*323.0*
PBW 175	*Indian*	*610*	*1.58*	*11.0*	*82.9*	*16.0*	*78*	*333.4*
Yitpi	AH	551	1.21	13.3	72.5	7.7	123	552.4
Fang	APW	537	1.22	12.6	74.5	8.8	126	602.8
Gladius	APW	529	1.05	12.7	71.3	9.3	142	471.4
Bumper	ASW	523	1.10	12.3	75.6	9.2	117	541.4
Kennedy	FEED	519	1.05	13.6	75.2	7.7	147	463.1
Calingiri	ASWN	473	0.88	12.2	65.2	4.2	134	499.9

LEGEND - **Ash**: ash content; **DS**: damaged starch content; **Ext**: dough extension; **FN**: falling number; **PSI0.9**: 90th percentile of the particle size distribution; **WA**: farinograph water absorption; **WP**: protein content

Table 4.10. Predicted means of selected flour quality traits for selected Australian wheat varieties from the Williams trial.

Wheat Variety	Grade	FN (sec)	Ash (%)	WP (%)	WA (%)	DS (%)	Ext (mm)	PSI0.9 (µm)
HI 1531	*Indian*	*677*	*1.30*	*11.8*	*86.0*	*14.5*	*85*	*323.0*
PBW 175	*Indian*	*610*	*1.58*	*11.0*	*82.9*	*16.0*	*78*	*333.4*
Yitpi	AH	419	1.06	10.4	66.9	8.2	113	734.1
Fang	APW	435	1.27	10.9	70.7	8.4	122	663.6
Gladius	APW	461	1.11	11.4	68.4	7.7	130	701.1
Bumper	ASW	461	1.06	10.1	71.4	8.3	103	682.5
Kennedy	FEED	456	1.14	12.0	73.8	8.0	119	683.5
Calingiri	ASWN	385	0.83	9.6	60.9	3.9	132	558.0

LEGEND - **Ash**: ash content; **DS**: damaged starch content; **Ext**: dough extension; **FN**: falling number; **PSI0.9**: 90th percentile of the particle size distribution; **WA**: farinograph water absorption; **WP**: protein content

The falling numbers of the Australian wheat varieties tested were all found to be greater than 300 seconds. The lowest falling number was 473 seconds for *Calingiri* from the Binnu trial and 357 seconds for *Tammarin Rock* from the Williams trial. It was observed that the wheat varieties in the Binnu trial generally had higher falling numbers than the wheat varieties in the Williams trial. In addition, there was a significant difference ($p<0.001$) in the falling number between the wheat varieties in the two trials. Overall, the grain of the Australian wheat varieties was of sound quality and the chapatti flours had low levels of α-amylase activity. Furthermore, in comparison to the Indian wheat varieties, there was no significant difference between the falling numbers of the Indian wheat varieties and the wheat varieties in the Binnu trial. There was however a significant difference ($p<0.001$) between the falling numbers of the Indian wheat varieties and the wheat varieties from the Williams trial. The level of α-amylase activity in the wheat varieties from the Williams trial was therefore greater than the Indian wheat varieties and also those from the Binnu trial; and this is likely to affect the starch quality of these samples (Anjum and Walker 2000). Genotype and environment both influence α-amylase activity and therefore the findings were not unexpected (Mares and Mrva 2008; MacArthur, D'Appolonia, and Banasik 1981).

The ash content of the wheat varieties in the Binnu and the Williams trials showed similar trends and were not found to be significantly different. The ash content of the hard wheat varieties in both trials ranged from 1.00 to 1.30%; and the ash content of the soft noodle wheat varieties were observed to be lower, ranging from 0.74 to 0.95%. The difference in ash content also indicated that there was less bran in the Australian soft noodle wheat chapatti flours (Symons and Dexter 1991). Wheat bran contains a significantly greater amount of ash than the endosperm as it increases in concentration outward from the centre of the grain; therefore ash content is routinely used as an indicator of bran contamination or flour purity (Kim and Flores 1999; Singh, Singh, and Singh Bakshi 1998). In contrast, the ash content of the Indian wheat varieties was significantly higher than the Australian wheat varieties ($p<0.001$). Therefore the Indian chapatti flours potentially contained more bran, average ash content 1.5%, than the Australian hard wheat chapatti flours produced as part of this research, which had an average ash content of 1.1%; so closer to the lower range reported for chapatti.

The bran content of flours has been reported to influence other flour quality traits, for example increased bran content was found to increase water absorption (Vetrimani, Sudha, and Haridas Rao 2005; Zhang and Moore 1997). In this research, however although it was thought that increased bran content increased water absorption, the ash content was not found to be significantly correlated to water absorption. The ash content was instead determined to have significant relationships with starch pasting properties and flour swelling volume. For example, ash content had a significant positive correlation with flour swelling volume and starch pasting breakdown for the Indian wheat varieties; but a significant negative correlation for these two quality traits for the Binnu hard wheat varieties. In addition, there was a significant positive relationship between ash content and starch pasting setback for the Binnu hard wheat varieties; and a significant positive correlation with starch pasting peak time for the Williams hard wheat varieties. Chen et al. (2011) also found similar relationships between bran content and starch pasting properties. The varied findings for the Indian and the Australian chapatti flours however indicated other factors, including the size of the bran particles, may have influenced flour quality traits affected by bran content. It was observed that the bran particles were generally smaller in size for the Indian wheat varieties, in comparison to the Australian wheat varieties, in this research. Contradictory findings in relation to the effects of bran content, have also been commented on in the literature, and it has been suggested that they are likely due to numerous other factors and influences; such as bran particle size (Noort et al. 2010).

Water absorption is also a key quality trait of chapatti flours due to its significant influence on chapatti quality. Chapatti is a steam leavened product and the appropriate amount of water allows steam to be formed at the end of baking; and facilitates the creation two layers, which is a defining characteristic of the product (Ghodke, Ananthanarayan, and Rodrigues 2009). Furthermore, the correct amount of water is required to produce dough of suitable consistency for kneading and sheeting. The range of water absorption values presented in Tables 4.9 and 4.10 are representative of the results for all of the Australian wheat varieties studied. The mean water absorption of the wheat varieties in the Binnu trial was 71.1% and 68.0% for the wheat varieties in the Williams trial. The mean water absorption of the Indian wheat varieties was 77.7%. Differences in water absorption between the three data sets were significant. The water absorption of the wheat varieties from the Binnu trial were significantly different to the Indian wheat varieties, and the wheat varieties from the Williams trial ($p<0.05$). In addition, the water absorption of the wheat varieties in the Williams trial were significantly lower than the water absorption of the Indian wheat varieties ($p<0.001$). The lower water absorption of the Australian chapatti flours, in contrast to the Indian chapatti flours, may be explained by the lower bran content of the flours, but also the lower damaged starch contents and differences in protein content. Water absorption was found to have significant positive correlations ($p<0.05$) with damaged starch content in the Binnu Hard, Binnu Soft and Williams Soft data sets; and a significant positive ($p<0.05$) relationship with protein content in the Williams Hard and Binnu Soft data sets.

The damaged starch content ranged from 3.9 to 9.3% for the Australian wheat varieties shown in Tables 4.9 and 4.10. The results for the Australian chapatti flours from the two trials were found to be similar and thus not significantly different. In contrast, the damaged starch content of the Australian chapatti flours were significantly lower than the Indian chapatti flours ($p<0.001$). A possible explanation for the significant differences in damaged starch content may be due to differences in grain hardness of the Indian and the Australian wheat varieties. Higher damaged starch is expected from hard wheats in comparison to soft wheats due to differences in the texture of the grain endosperm. Hard wheats have greater tendency to produce coarser particles from milling and fracture in a way that results in more broken starch granules and consequently more damaged starch; whereas soft wheats are more easily fractured leaving a greater number of intact starch granules after milling (Pasha, Anjum, and Morris 2010; Morris 2002; Turnbull and Rahman 2002). Furthermore, it was observed that the Australian soft noodle wheat chapatti flours had lower damaged starch contents, 4.1% and 3.6%, than the Australian hard wheat varieties, 8.1% and 7.6%, from the Binnu and the Williams trials; respectively. High damaged starch is primarily important in chapatti flours to facilitate greater water absorption. The damaged starch content also contributes to the sweetish taste and baked appearance of chapatti through Maillard browning reactions.

Wheat protein content is an important quality trait which influences both dough and end product quality. The protein content of the Australian wheat varieties grown in the Binnu trial ranged from 12.2 to 13.6%; as shown in Table 4.9. In the Williams trial, the protein content of the Australian wheat varieties ranged from 9.6 to 12.0% and selected results are presented in Table 4.10. The protein content of the wheat varieties in the two trials was determined to be significantly different ($p<0.001$). The wheat varieties from the Binnu trial had higher protein contents, than the wheat varieties from the Williams trial. One of the effects of protein content was shown by the higher water absorption of the chapatti flours from the Binnu trial, in comparison to the chapatti flours from the Williams trial. Protein content is known to affect water absorption; with higher protein content generally correlated to higher water absorption (Wheat Marketing Centre Inc. 2008; Ma et al. 2007). Lastly, the protein content of the wheat varieties in the Binnu trial was significantly higher than the Indian wheat varieties.

The quality trait of dough extension ranged from 117 to 154 mm for the wheat varieties from the Binnu trial and 103 to 150 mm for the wheat varieties from the Williams trial. The values for extension, as shown in Tables 4.9 and 4.10, reflect these results; however a significant difference was determined between the dough extension of the wheat varieties from the Binnu trial, in comparison to the Williams trial ($p<0.001$). Higher values of extension were determined for the Australian wheat varieties from the Binnu trial, and indicated that the dough was able to be stretched a greater distance. The wheat varieties in the Binnu trial also had greater protein contents and therefore this was likely to have contributed to this finding. In addition, the Australian wheat varieties were significantly different ($p<0.001$) to the Indian wheat varieties, which had significantly lower values for dough extension; hence less extensible dough. The differences in dough rheology of the Australian and the Indian wheat varieties suggested differences in protein quality, particularly HMW-GS and LMW-GS compositions.

The 90th percentile of the particle size distribution of a flour sample analysed has been shown for selected Australian wheat varieties in Tables 4.9 and 4.10. The difference between the minimum and the maximum particle size indexes of the wheat varieties in the Binnu trial and the Williams trial were similar, 186.4 µm and 203.3 µm; respectively. However, although the ranges were comparable in value, the particle size results for the wheat varieties in the two trials were significantly different ($p<0.001$). Generally, the particle sizes of the chapatti flours in the Williams trial were larger than those in the Binnu trial. The differences in particle size could be due to environmental factors such as moisture content (Muhamad and Campbell 2004; Morris 2002). However as the genotypes were the same in both trials, except for one wheat variety *Peake* in the Williams trial, a more probable explanation for particle size differences was the milling conditions. It was later observed that over time, despite the aperture setting not being changed on the laboratory stone mill, the distance between the stones may have slightly increased and this was likely to have resulted in the larger particle size of the chapatti flours from the Williams trial. In addition, the particle sizes of the Australian chapatti flours from the two trials were significantly greater than the particle sizes of the Indian chapatti flours studied ($p<0.001$). The mean particle size of the 90th percentile of the particle size distribution of the Indian wheat varieties was 339.0 µm, compared to 519.0 µm and 672.2 µm for the Australian wheat varieties in the Binnu and the Williams trials; respectively. It was interesting to discover the hard Indian wheat varieties also had finer particle size flour than the Australian soft noodle wheat varieties. Grain hardness is known to be genetically controlled by the hardness locus on the short arm of chromosome 5D, by two closely linked genes puroindoline a (*Pina-D1*) and puroindoline b (*Pinb-D1*); and can be measured by particle size index (Lillemo et al. 2006; Morris 2002). It has been reported that Australian wheat varieties are mainly limited to the two major types of genetically determined grain hardness combinations (Pickering and Bhave 2007). Whereas, wheat landraces including Indian wheat varieties have more diverse genetic expressions for grain hardness thus allowing for different degrees of grain hardness and endosperm texture (Pickering and Bhave 2007; Lillemo et al. 2006; Ram et al. 2005b). The finding also helps to further clarify the wheat quality requirement reported for chapatti making of medium hard wheat (Nagarajan 2006).

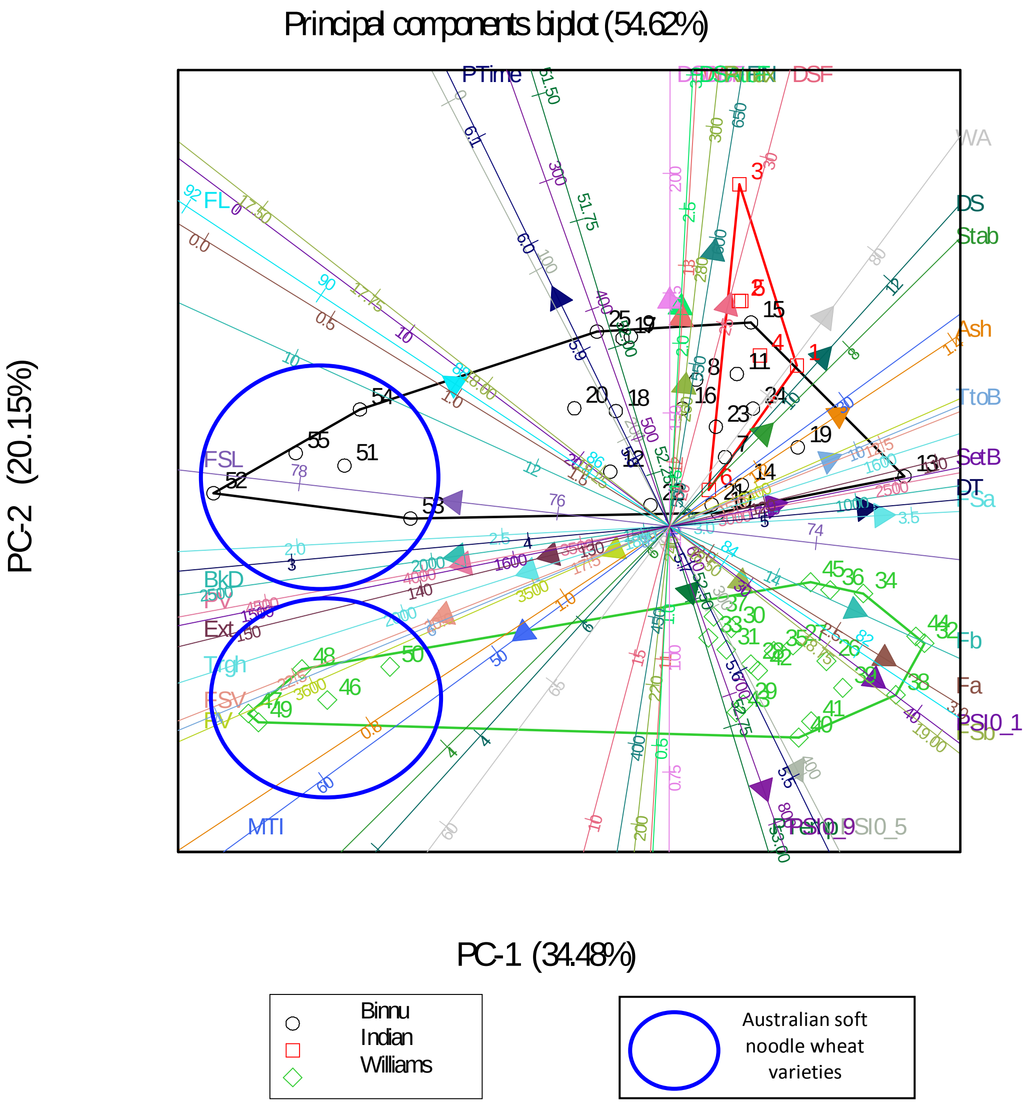
The selected flour quality traits presented in Tables 4.9 and 4.10 represent some of the traditionally important quality characteristics of chapatti flour plus some new quality traits of interest from this research. Analysis of the results for these quality traits highlighted significant differences in the quality of the chapatti flours produced from the Indian and the Australian wheat varieties.

Comparison of Indian and Australian chapatti flour quality

Principle component analysis (PCA) was performed to gain an understanding of the similarities between the Indian and the Australian chapatti flours based on all of the flour quality traits measured. PCA determined 54.62% of the variation between the wheat varieties, based on all of the flour quality traits measured, was mainly being driven by principle component one (PC-1) which accounted for 34.48% of the variation; and could also be explained by principle component two (PC-2) which was determining 20.15% of the variation between the wheat varieties. The PCA output can be referred to in Appendix 1.26. A biplot, Figure 4.4, was generated to visualise relationships between the flour quality traits and the wheat varieties. A convex hull is depicted on the biplot and encloses the wheat varieties in each data set. The Binnu wheat varieties are in black, the Williams wheat varieties in green, and the Indian wheat varieties in red. The Australian soft noodle wheat varieties in the Binnu and the Williams trials have been circled in blue.

Differences in chapatti flour quality of the Australian wheat varieties from the Binnu trial and the Williams trial were previously identified. Figure 4.4 further confirmed these differences, as shown by the different position of the wheat varieties from the Binnu trial on the biplot, in comparison to the wheat varieties from the Williams trial. The wheat varieties from both of the trials are shown to be spread across the biplot however the Binnu trial (black) is in a higher position than the wheat varieties from the Williams trial (green). The second principle component (PC-2) was largely responsible for explaining differences in flour quality between these two data sets. The chapatti flours from the Binnu trial had higher values for falling number, dough stickiness properties, starch pasting peak time, protein content and water absorption; and lower values for particle size index than the chapatti flours from the Williams trial. The differences in chapatti flour quality were expected due to the effect environment and GxE interactions have on flour quality traits; and were used to help understand the ability of Australian wheat varieties to make chapatti.

Figure 4.4. Biplot of PCA of flour quality traits for the Indian and the Australian wheat varieties.



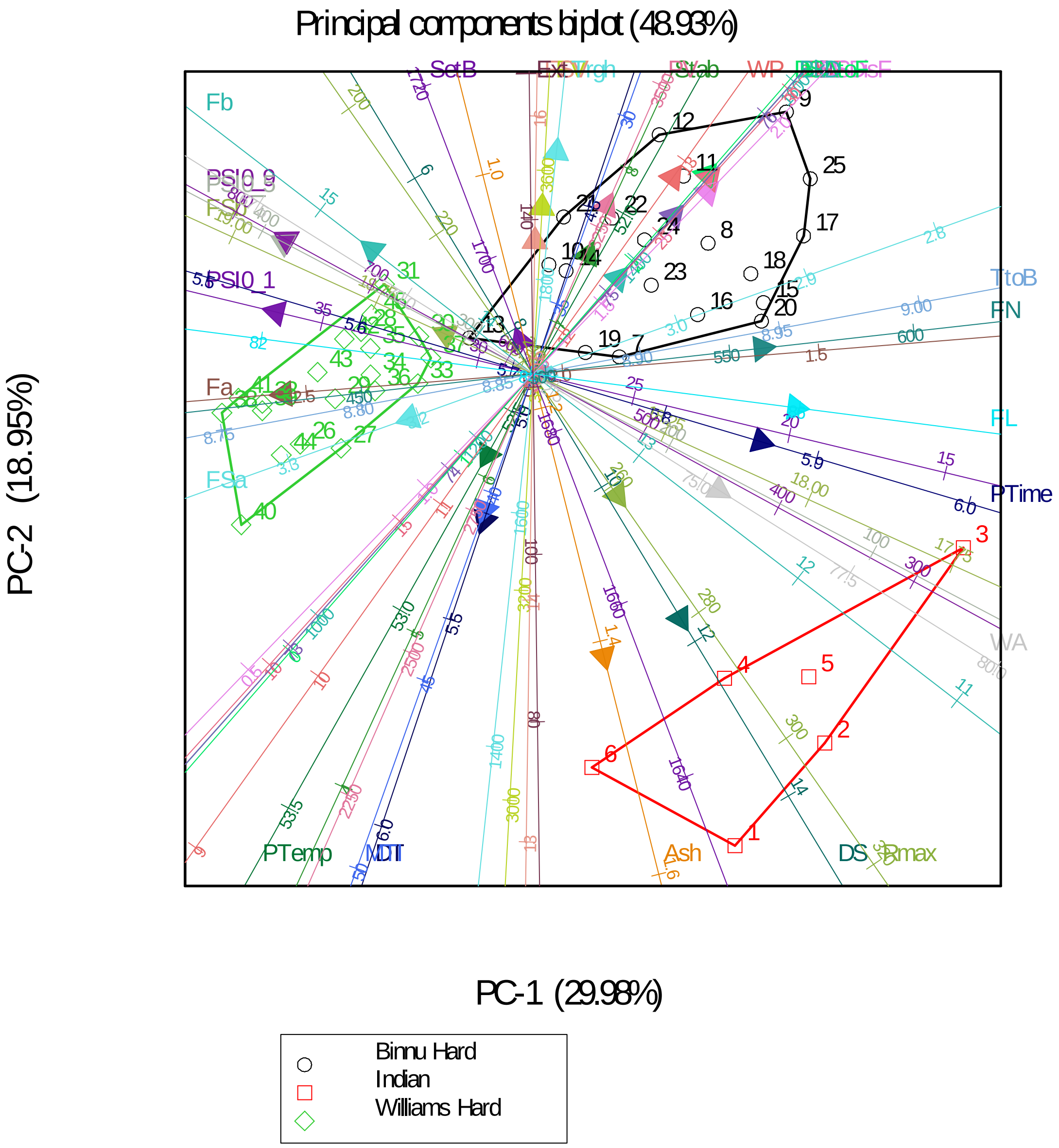
In addition, the flour quality of the Australian soft noodle wheat varieties was observed to be different in comparison to the Indian and the Australian hard wheat varieties. The Australian soft noodle wheat varieties from both trials are shown to be clustered on the left hand side of the biplot, Figure 4.4, and are circled in blue. Conversely, the Indian and the Australian hard wheat varieties are positioned on the right hand side of the biplot, indicating different flour quality. The first principal component (PC-1) mainly explained the differences between the hard and soft wheat varieties and the flour quality traits of; flour colour *CIE L** and *a**, starch pasting peak viscosity and breakdown, flour swelling volume and particle size of the 10th percentile of the particle size distribution, were identified as being important. The Australian soft noodle wheat varieties were characterised as having higher starch swelling and peak viscosity, which was expected as they have more intact starch granules than hard wheat varieties (Khan and Shewry 2009). Also the Australian soft noodle wheat varieties had higher *CIE L**, lightness, and lower *a**, redness, values as there was less bran and more endosperm in these flour samples because of the way soft wheat types mill (Khan and Shewry 2009; Turnbull and Rahman 2002). The research sought to select wheat varieties with potential for chapatti making and the differences provided a range of quality types for defining what is needed for chapatti quality. The chapatti flour quality results suggested that the flour quality of the Australian hard wheat varieties was more comparable to the flour quality of the Indian wheat varieties, than the Australian soft noodle wheat varieties.

PCA also included the Indian wheat varieties and these can be seen on the biplot, Figure 4.4, represented in red. The Australian hard wheat varieties; *Bumper*, *Carnamah*, *Correll*, *EGA Wentworth*, *Fang*, *Gladius*, *Guardian*, *Magenta*, *Westonia*, *Yitpi* and *Young* from the Binnu trial were shown to have similarities in chapatti flour quality with the Indian wheat varieties. Figure 4.4 shows clustering of the Indian wheat varieties (red), except for point number three, *HI 1531*, with eleven of the Australian hard wheat varieties from the Binnu trial (black). The similar positioning of these wheat varieties suggests similarities in the values for important flour quality traits. Nonetheless, because the previous finding showed the Australian soft noodle wheat varieties were dissimilar to the Indian and the Australian hard wheat varieties, another PCA was needed which excluded the Australian soft noodle wheat varieties from the analysis.

A second PCA was performed on the flour quality data of the Indian and the Australian hard wheat varieties only. Appendix 1.27 describes the PCA output and latent vector loadings. The chapatti flour quality of the wheat varieties from the Binnu trial, the wheat varieties from the Williams trial, and the wheat varieties from

India were all shown to have different flour quality. The biplot from this analysis, Figure 4.5, illustrates the flour quality differences between the three data sets; the Indian wheat varieties (red), the Australian hard wheat varieties from the Binnu trial (black), and the Australian hard wheat varieties from the Williams trial (green). The location of the wheat varieties on the biplot changed, in comparison to Figure 4.4, because the range for each of the flour quality traits was changed by the removal of the Australian soft noodle wheat varieties.

Figure 4.5. Biplot of PCA of flour quality traits for the Indian and the Australian hard wheat varieties.



The first principal component was determined to account for 29.98% of the variation between wheat varieties; and falling number, flour colour *CIE L** and *a**, particle size index, starch pasting peak time and water absorption were important quality traits. PC-1 was mainly separating the wheat varieties from the Williams trial, from the Indian wheat varieties and the wheat varieties from the Binnu trial. The wheat varieties from the Williams trial can be seen on the left hand side of Figure 4.5, whereas the remaining wheat varieties are on the right hand side of the biplot. The wheat varieties from the Williams trial generally had lower values for falling number, flour colour *CIE L**, lightness, and starch pasting peak time; and higher values for flour colour CIE a*, redness, and particle size index.

The second principal component explained 18.95% of the variation between the wheat varieties and the quality traits of ash content, damaged starch content, dough extension and starch pasting minimum viscosity (trough) were significant contributing factors. PC-2 was largely differentiating the Australian hard wheat varieties from the Indian wheat varieties; as shown by the higher position of the Australian hard wheat varieties (black and green) on the biplot in contrast to the Indian wheat varieties (red) which can be seen clustered towards the bottom of the biplot. The Indian wheat varieties in comparison to the Australian hard wheat varieties had lower values for dough extension and starch pasting minimum viscosity; and higher values for ash content and damaged starch content. These determinations further confirmed the previous findings of significant differences between the Indian and the Australian hard wheat varieties for selected flour quality traits.

Quality traits determining differences in chapatti flour quality

PCA was conducted to understand the combinations of flour quality traits that were determining differences between the wheat varieties in each data set. The wheat varieties were grouped into the data sets: Indian, Binnu Hard, Binnu Soft, Williams Hard and Williams Soft. The ‘hard’ and ‘soft’ referred to the Australian hard wheat varieties and the Australian soft noodle wheat varieties studied; respectively. The first principal component (PC-1) for each of the data sets is presented in Table 4.11 and the latent vector loadings for each flour quality trait are shown. Appendix 1.28 contains the PCA output for each of the data sets. The flour quality traits of importance, in the first principal component, have been highlighted in blue in Table 4.11; and indicated that each data set had a different combination of quality traits determining differences between the wheat varieties in that data set.

Two quality traits, flour slurry colour *CIE a** (FSa) and starch pasting peak time (PTime) however were found to be important in more than one data set; see Table 4.11. The quality trait, flour slurry colour *CIE a**, indicating redness, was important for determining differences between the Australian soft noodle wheat varieties from the Binnu and the Williams trials. Starch pasting peak time (PTime) was identified as an important quality trait in the Binnu Hard and the Williams Soft data sets. In addition, although dough development time (DT) and dough time to breakdown (TtoB) were also highlighted as important quality traits in two different data sets, the Indian and the Binnu Soft, the direction of the latent vectors was positive in one of the data sets and negative in the other. Therefore the flour quality traits had different relationships with the other flour quality traits; positive correlations in the Indian data set but negative correlations in the Binnu Soft data set. It may be concluded that the flour quality for each set of wheat varieties was unique and the differences seen in Figures 4.4 and 4.5 were further confirmed from the PCA analyses conducted on each data set. Thus the flour quality traits differentiating the wheat varieties in each data set were different; and this is most likely due to differences in genotype, environment, and their interactions on flour quality.

Table 4.11. Table of the first principle component (PC-1) of PCA correlation matrix analysis for flour quality traits.

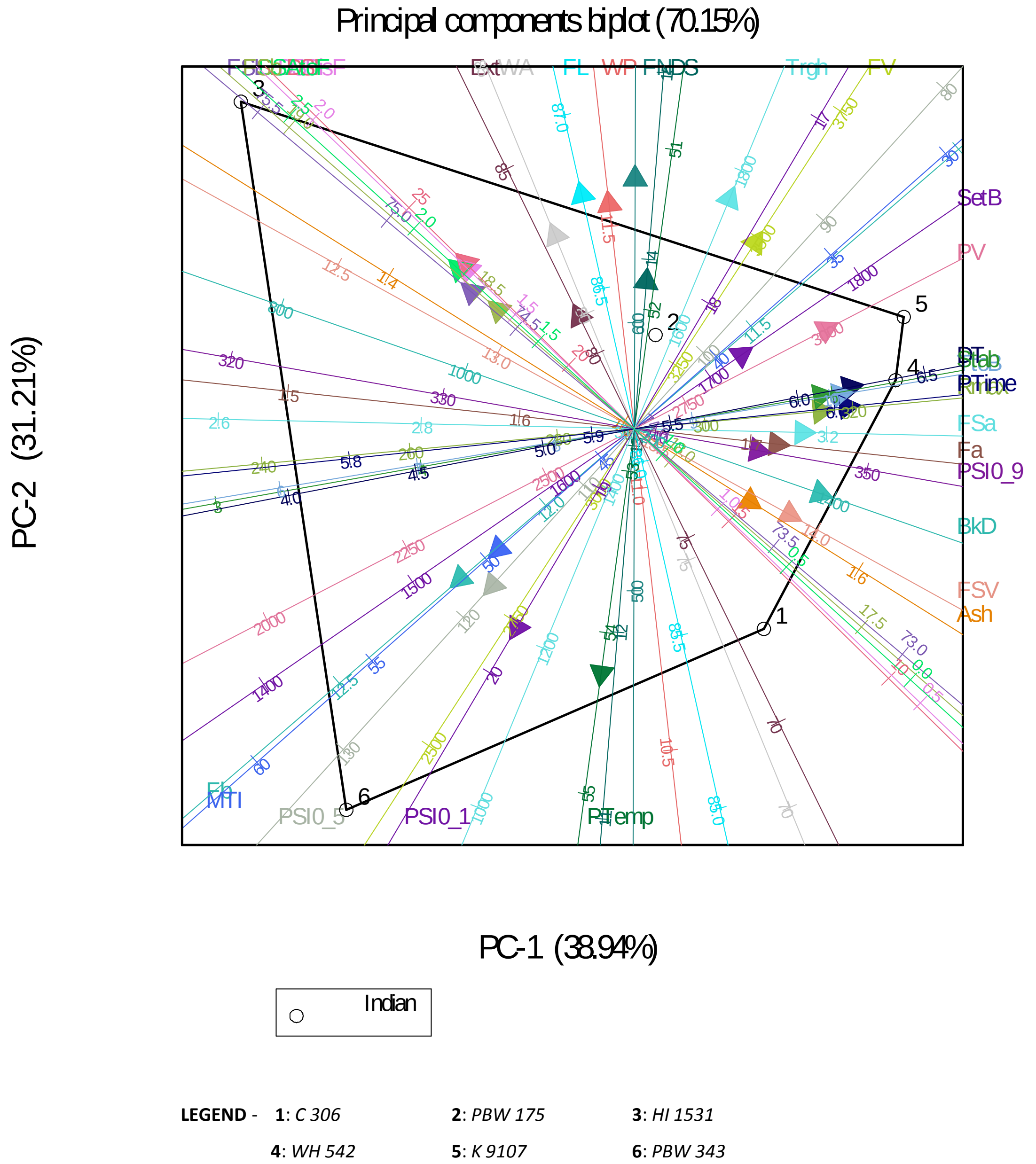
Trait or Latent vectors[1]	PC-1 Loadings				
	Indian	Binnu Hard	Williams Hard	Binnu Soft	Williams Soft
Ash	0.144	0.253	0.044	-0.191	-0.210
BkD	0.232	-0.187	0.074	0.225	0.177
DS	0.015	-0.009	0.154	-0.098	-0.221
DSAtoF	-0.220	-0.140	-0.244	0.161	0.112
DSDisF	-0.208	-0.155	-0.238	0.201	0.120
DSF	-0.211	-0.112	-0.236	0.095	0.094
DT	0.270	0.027	0.166	-0.244	0.036
Ext	-0.069	-0.194	-0.255	0.181	0.182
FL	-0.066	-0.300	0.046	0.217	0.209
FN	0.001	0.145	0.289	0.085	0.077

FSL	-0.204	-0.180	0.059	0.191	0.216
FSV	0.194	-0.245	0.039	0.213	-0.033
FSa	0.210	0.127	-0.090	-0.252	-0.243
FSb	-0.170	0.224	0.133	0.144	0.196
FV	0.151	0.182	0.294	0.131	0.217
Fa	0.178	0.244	-0.126	-0.243	-0.239
Fb	-0.218	0.262	0.123	-0.099	0.198
MTI	-0.170	-0.062	-0.143	0.219	-0.216
PSI0.1	-0.147	0.278	-0.078	-0.166	0.060
PSI0.5	-0.177	0.270	-0.124	-0.151	0.156
PSI0.9	0.154	0.248	-0.162	-0.259	0.151
PTemp	-0.042	0.101	-0.103	0.171	-0.160
PTime	0.266	-0.254	0.224	0.132	-0.241
PV	0.240	-0.146	0.177	0.196	0.180
Rmax	0.233	-0.022	0.205	-0.055	0.037
SetB	0.134	0.228	0.184	0.050	0.258
Stab	0.233	0.001	0.166	-0.213	0.212
Trgh	0.119	0.040	0.297	0.151	0.170
TtoB	0.257	0.053	0.168	-0.255	0.188
WA	-0.099	-0.004	-0.154	-0.169	-0.254
WP	-0.032	0.075	-0.259	-0.106	0.110
Number of wheat varieties	**6**	**19**	**20**	**5**	**5**
Principal component 1 (%)	**38.94**	**30.53**	**24.71**	**45.11**	**42.19**

[1]**LEGEND** - **Ash**: ash content; **Bkd**: starch pasting breakdown; **DS**: damaged starch content; **DSAtoF**: dough stickiness area under the curve to peak force; **DSDisF**: dough stickiness distance to peak force; **DSF**: dough stickiness peak force; **DT**: dough development time; **Ext**: dough extension; **FL**: flour colour *CIE L**; **FN**: falling number; **FSL**: flour slurry colour *CIE L**; **FSV**: flour swelling volume; **FSa**: flour slurry colour *CIE a**; **FSb**: flour slurry colour *CIE b**; **FV**: starch pasting final viscosity; **Fa**: flour colour *CIE a**; **Fb**: flour colour *CIE b**; **MTI**: farinograph mixing tolerance index; **PSI0.1**: 10th percentile of the particle size distribution; **PSI0.5**: 50th percentile of the particle size distribution; **PSI0.9**: 90th percentile of the particle size distribution; **PTemp**: starch pasting temperature; **PTime**: starch pasting peak time; **PV**: starch pasting peak viscosity; **Rmax**: dough maximum resistance to extension; **SetB**: starch pasting setback; **Stab**: farinograph dough stability; **Trgh**: starch pasting trough; **TtoB**: farinograph dough time to breakdown; **WA**: farinograph water absorption; **WP**: protein content.

Nonetheless, further understanding of the flour quality traits determining differences between the Indian wheat varieties may be of importance if they have relationships with chapatti quality. The first principal component (PC-1) explained 38.94% of the variation and was mainly driven by the traits of dough development time, starch pasting peak time and dough time to breakdown. PC-1 appeared to be separating the Indian wheat varieties with better chapatti making quality, *HI 1531* and *PBW 175*, towards the left hand side of the biplot, Figure 4.6; and these wheat varieties had lower values for dough development time, dough time to breakdown, and starch pasting peak time. The dough of these wheat varieties therefore required less time to develop, had faster breakdown, and starch pasting peak viscosity was also reached faster; this possibly indicates dough strength is not important for chapatti quality, and potentially confirms the importance of high damaged starch.

Figure 4.6. Biplot of PCA of flour quality traits for the Indian wheat varieties.



The second principal component explained 31.21% of the differences in flour quality, between the Indian wheat varieties, and was largely determined by falling number, starch pasting temperature, flour colour *CIE L**, starch pasting trough or minimum viscosity, and protein content. The Indian wheat varieties with good chapatti quality, *HI 1531* and *PBW 175*, were positioned at the top centre to left of the biplot, Figure 4.6, and had higher values for falling number, protein content, flour colour *CIE L**, lightness, and starch pasting minimum viscosity, but lower values for starch pasting temperature. If the Indian wheat varieties have different chapatti quality, as has been reported, then the flour quality traits described may be of importance as additional indicators of chapatti quality. The potential of wheat lines and varieties to make quality end products needs to be identified as early as possible and further understanding of the wheat quality requirements for chapatti is important for this.

Characterisation of the chapatti quality of the Indian wheat varieties

Chapatti quality was evaluated by measurement of the following quality traits and included; percentage bake loss, puffed height, percentage surface area of the chapatti puffed, chapatti dough and baked colour measurements, and the measurement of textural attributes using two different extensibility tests. Predicted means for each of the chapatti quality traits were determined for the Indian wheat varieties and have been described in Appendix 1.29. Table 4.12 outlines the predicted means for percentage bake loss, puffed height and percentage surface area of the chapatti puffed of the Indian wheat varieties. Percentage bake loss quantified the loss of moisture from the chapatti before and after baking and was shown to range from 14.3 to 18.5%. Indian wheat variety *HI 1531* had the greatest loss of moisture and *WH 542* had the lowest percentage bake loss. The variable loss of moisture due to baking was related to differences in flour water absorption of the Indian wheat varieties; and a significant positive correlation between bake loss and water absorption was identified ($p<0.05$). Differences in the percentage bake loss between the Indian wheat varieties however were determined not to be significant.

The puffed height of chapatti was measured from the outer lower surface of the chapatti to the outer top surface of the chapatti; and ranged from 39.0 to 55.3 mm. Puffed height is an important quality characteristic of chapatti, as puffing is responsible for creating two distinct layers, which distinguishes it from other flat unleavened Indian breads such as tandoori roti (Saxena, Salimath, and Haridas Rao 2000). Indian wheat variety *C 306* had the lowest predicted mean for puffed height of 39.0 mm and *HI 1531* had the highest puffed height of 55.3 mm. The puffed height values obtained were similar to those reported in the literature for chapatti made from Indian wheat varieties. Srivastava, Prasada Rao, and Haridas Rao (2003) found that the puffed height ranged from 44 to 66 mm; Hemalatha et al. (2007) reported the chapatti puffed height ranged from 47 to 52 mm; and Prabhasankar, Manohar, and Gowda (2002) determined the puffed height to range from 45 to 57 mm. A chapatti puffs as a result of steam formed during baking, from water in the dough sheet. The puffed height and percentage bake loss, or water absorption, however were not significantly correlated. Puffed height was instead found to have significant positive correlations with falling number, flour colour *CIE L**, dough stickiness peak force, dough extension, protein content and starch pasting trough; and a significant negative correlation was identified with starch pasting temperature. The correlations signified that protein content and dough properties were also of importance for chapatti puffing; and this may have implications on chapatti texture and eating quality. The measurement of puffed height was found to be effective in discriminating differences in chapatti quality between the Indian wheat varieties ($p<0.001$).

Table 4.12. Predicted means for the chapatti quality traits of percentage bake loss, puffed height and percentage surface area of the chapatti puffed, of the Indian wheat varieties.

Wheat Variety	BL (%)	PH (mm)	SAP (%)
HI 1531	18.5	55.3	100.0
PBW 175	16.1	50.6	99.2
C 306	16.7	39.0	88.7
K 9107	15.4	50.6	96.9
PBW 343	15.9	42.1	90.4
WH 542	14.3	47.5	96.2

LEGEND - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed

In addition, the complete and full puffing of chapatti is important as it assists in assessing if two separate layers have been properly created; and having two layers is a defining attribute of this end product. The percentage surface area of the chapatti puffed therefore measured the ability of a chapatti to puff across its entire surface area. Indian wheat variety *HI 1531* puffed completely across the surface and wheat variety *C 306* puffed across 88.7% of the chapatti's surface area; see Table 4.12. The remaining Indian wheat varieties had chapattis which puffed across greater than 90% but less than 100% of the chapatti's surface area. It is desirable for complete, 100%, puffing across the entire surface area of the chapatti, however greater than 90% was also regarded as being of good quality. In addition, the percentage surface area puffed was found to have significant positive correlations to puffed height and a number of the flour quality traits that puffed height was also significantly correlated with; such as falling number, flour colour *CIE L**, protein content, starch pasting trough, and a significant negative correlation with starch pasting temperature. Differences observed between the Indian wheat varieties based on puffing ability were determined to be significant ($p<0.05$). The formation of two distinct layers is a key characteristic of chapatti and a quality attribute which differentiates it from other traditional Indian flat unleavened breads.

Chapatti should also have an appealing appearance and this includes colour to the consumer; a light creamish brown colour has been commonly reported for good quality chapatti (Hemalatha et al. 2007; Revanappa, Bhagwat, and Salimath 2007). *CIE L* a** and *b** colour measurements were taken on both sides of the chapatti for the raw chapatti dough sheet and the baked chapatti. Table 4.13 contains the predicted means for the raw chapatti dough sheet colour measurements of the Indian wheat varieties. The colour readings taken on side one and on side two of the raw chapatti dough sheets were shown not to be significantly different; and this was expected as the dough sheet was rolled from one homogenous piece of dough. *CIE* colour values for side one and side two ranged from 68.33 to 72.81 for *CIE L**; 4.70 to 6.21 for *CIE a**; and 20.80 to 24.86 for *CIE b**. It was observed that Indian wheat variety *HI 1531* had the lightest colour dough sheet, with the highest *CIE L** value; the lowest *CIE a** value, indicating lower redness; and one of the higher *CIE b** values, representing increased intensity of yellow colour. The Indian wheat varieties

C 306 and *WH 542*, in contrast, were determined to have the darkest colour dough sheets, with the lowest *CIE L** values of the Indian wheat varieties tested. Furthermore, the results indicated *WH 542* had browner colour dough sheets with high *CIE a** and *b** values in comparison to the other wheat varieties; see Table 4.13. The darkening of chapatti dough, particularly during the dough resting period, has been reported as being detrimental and negatively affecting chapatti colour; enzyme activities of polyphenol oxidases and peroxidases have been identified as contributing factors (Yadav et al. 2008c; Hemalatha et al. 2007).

Table 4.13. Predicted means for raw chapatti dough sheet colour measurements of the Indian wheat varieties.

Wheat Variety	R1L (*L**)	R1a (*a**)	R1b (*b**)	R2L (*L**)	R2a (*a**)	R2b (*b**)
HI 1531	72.81	4.70	24.07	72.64	4.73	24.27
PBW 175	70.49	5.56	24.51	70.28	5.65	24.86
C 306	68.57	5.09	20.80	68.33	5.23	21.24
K 9107	69.59	5.83	23.48	69.36	5.90	23.91
PBW 343	70.34	5.05	23.66	69.99	5.20	23.94
WH 542	68.47	6.16	24.27	68.43	6.21	24.58

LEGEND - **R1L**: raw chapatti dough sheet colour *CIE L** of side one; **R1a**: raw chapatti dough sheet colour *CIE a** of side one; **R1b**: raw chapatti dough sheet colour *CIE b** of side one; **R2L**: raw chapatti dough sheet colour *CIE L** of side two; **R2a**: raw chapatti dough sheet colour *CIE a** of side two; **R2b**: raw chapatti dough sheet colour *CIE b** of side two

As previously described, chapatti colour is a major contributing factor to end product appearance and an attractive appearance is necessary for consumer acceptance. The change in colour from chapatti dough to the baked chapatti was investigated to assess the impact dough colour has on the end product. The percentage change in the colour of the chapatti, before and after baking, was calculated and the results are displayed in Table 4.14. The percentage change in *CIE L** values after baking were shown to vary between the wheat varieties. For half of the Indian wheat varieties, *HI 1531*, *PBW 175* and *PBW 343*, the lightness of the chapatti generally decreased after baking shown by positive values. Conversely, for the other wheat varieties, *K 9107*, *WH 542* and *C 306*, the lightness generally increased as shown by the negative $\%\Delta L^*$ values in Table 4.14. Overall the percentage change in *CIE L** values due to baking ranged from -0.99 to 1.98%. One factor which may have affected the *CIE L** readings was dusting of the dough sheet with flour for ease of rolling. Nonetheless, it may be concluded that the change in lightness due to baking varied and the differences observed between wheat varieties were found to be significant ($p<0.001$) and most likely due to differences in genotype.

Table 4.14. Predicted means for the percentage change in chapatti colour, from raw to baked, of the Indian wheat varieties.

Wheat Variety	%Δ1L*	%Δ1a*	%Δ1b*	%Δ2L*	%Δ2a*	%Δ2b*
HI 1531	1.36	17.69	1.79	1.98	11.61	3.13
PBW 175	-0.07	14.68	4.37	1.21	7.90	3.94
C 306	-0.53	9.59	-1.11	-0.56	8.85	2.02
K 9107	0.01	9.38	-0.04	-0.43	10.03	4.81
PBW 343	1.71	6.29	0.00	1.14	7.13	3.51
WH 542	-0.99	13.66	2.43	0.53	6.23	1.75

LEGEND - **%Δ1L***: percentage change in chapatti colour from raw to baked of *CIE L** of side one; **%Δ1a***: percentage change in chapatti colour from raw to baked of *CIE a** of side one; **%Δ1b***: percentage change in chapatti colour from raw to baked of *CIE b** of side one; **%Δ2L***: percentage change in chapatti colour from raw to baked of *CIE L** of side two; **%Δ2a***: percentage change in chapatti colour from raw to baked of *CIE a** of side two; **%Δ2b***: percentage change in chapatti colour from raw to baked of *CIE b** of side two

The percentage change in *CIE a** values after baking showed a consistent positive trend for each of the Indian wheat varieties and ranged from 6.23 to 17.69%. The positive change in *CIE a** values represented a decrease in redness after baking and this was observed as having a positive effect on baked chapatti colour. The percentage change in *CIE a** was found to be significant for side one ($p<0.001$) and side two ($p<0.05$) between the Indian wheat varieties tested. Furthermore, the effect of baking on *CIE b** was also shown to generally decrease in value, particularly for side two of the chapatti, and indicated a decrease in yellowness. However, for Indian wheat variety *C 306*, the percentage change in *CIE b** values was shown to increase for side one of the chapatti; and largely remained unchanged for wheat varieties *K 9107* and *PBW 343*. The differences observed between the Indian wheat varieties for the percentage change in *CIE b** for side one were significant ($p<0.001$) however were not significant for side two. The shorter baking time for side two of the chapatti, in comparison to side one, which had a longer baking time, was the most probable explanation.

Overall the changes in chapatti colour of the Indian wheat varieties due to baking were not large. The chapattis mostly became lighter in brown colour due to a decrease in redness, *CIE a** value, and yellowness, *CIE b** value. The change in overall lightness of the chapattis was variable, *CIE L** values, but not greatly different to the raw dough sheet. Hatcher, Kruger and Dhaliwal (1997) also found raw chapattis were darker, redder and yellower than baked chapattis. The chapatti colour differences between Indian wheat varieties were significant, indicating an effect of genotype (Mares and Campbell 2001). Chapatti which has a light creamy brown, to sometimes golden colour, has been reported to be preferred, however too pale or too dark colour is not acceptable (Hemalatha et al. 2007; Gill, Sodhi, and Kaur 2005; Hatcher, Kruger, and Dhaliwal 1997).

Chapatti texture has been described as needing to be soft and pliable; thus making it easy to tear, fold and form a scoop for eating with curries (Ur-Rehman, Paterson, and Piggott 2006; Prabhasankar, Manohar, and Gowda 2002). The texture should not be tough, leathery or brittle (Gill, Sodhi, and Kaur 2005). The textural attributes of the chapattis were assessed using two different extensibility tests performed using the texture analyser (Stable Microsystems) and the results are shown in Table 4.15. The peak force required to tear a chapatti strip (E1PF) made from the Indian wheat varieties was shown to range from 484 to 766 g; with the higher the peak force, the tougher the chapatti strip as it required greater force to tear. Indian wheat variety *PBW 175* required the lowest peak force to tear and wheat variety *C 306* the highest peak force to tear. Therefore the texture of Indian wheat variety *C 306* was tougher than *PBW 175* as it had a greater resistance to tearing; which is not desirable for eating.

Hand tearing of chapatti is performed when chapattis are consumed and extensibility has been thought to capture and help assess the textural traits of softness, tearing ease and pliability (Ghodke and Ananthanarayan 2007; Gujral and Gaur 2002). If there are low values for extensibility this may indicate brittle, possibly soft, inelastic texture; however very high values of extensibility can be evidence of tough chapatti or highly elastic texture as it is able to be stretched further. The extensibility of a chapatti strip was measured as the distance to peak force (E1Dis) and represented the length the chapatti strip was stretched to before it tore. The distance to peak force ranged from 4.61 to 5.83 mm; and Indian wheat variety *WH 542* was determined to be the least extensible and wheat variety *PBW 175* the most extensible of the Indian wheat varieties tested. The last attribute quantified was the work required to extend and tear a chapatti strip, measured as the area under the curve to peak force (E1A12). The work required to tear a chapatti strip ranged from 1936.48 to 2836.81 gs^{-1}; with Indian wheat variety *PBW 175* requiring the least amount of work, and wheat variety *PBW 343* the greatest amount of work, to tear a chapatti strip. Differences between Indian wheat varieties for the parameters of E1PF ($p<0.001$) and E1A12 ($p<0.05$) were both found to be significant; the E1Dis values were not determined to be significant.

Table 4.15. Predicted means for the texture measurements taken from two extensibility tests for the Indian wheat varieties.

Wheat Variety	E1PF (g)	E1Dis (mm)	E1A12 (g.s)	E2PF (g)	E2Dis (mm)	E2A12 (g.s)
HI 1531	587.76	5.36	1969.22	814.13	23.22	5854.76
PBW 175	484.18	5.83	1936.48	800.93	21.37	6231.90
C 306	766.21	4.86	2654.90	939.58	20.97	7126.57
K 9107	677.27	4.75	2309.24	1001.58	19.70	6718.23
PBW 343	704.83	5.42	2836.81	981.72	20.26	6837.79
WH 542	688.15	4.61	2188.77	801.05	19.17	5226.29

LEGEND - **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti

A second extensibility test was also conducted and was used as an alternative method to further characterise chapatti textural attributes of softness, tearing ease and pliability, through extensibility measurements. The second extensibility test used a different probe attachment and method, but also quantified textural attributes by measuring parameters of peak force, distance to peak force and area under the curve to peak force. These measurements represented the force required to puncture a chapatti, the distance the chapatti stretched until puncturing, and the work required to puncture the chapatti sample. The peak force required to puncture a whole chapatti (E2PF) was found to range from 800.93 to 1001.58 g. Indian wheat variety *PBW 175* required the least amount of force to puncture a chapatti, and wheat variety *K 9107* the greatest amount of force to puncture a chapatti. The greater the force required to puncture or rupture the chapatti, the tougher the texture. The findings for peak force, although positively correlated to the peak force results from the first extensibility test, were not significantly correlated. One consistent finding however was shown for wheat variety *PBW 175* which was identified as requiring the least amount of force to both tear and puncture, indicating a softer texture chapatti. In addition, the peak force to puncture a chapatti (E2PF) was found to have a significant positive correlation to the work required to tear a chapatti strip in the first extensibility test (E1A12). Differences between the Indian wheat varieties for the peak force required to puncture a whole chapatti were significant ($p<0.001$).

The second measurement, distance to peak force (E2Dis) measured the distance the centre of a chapatti could be extended to until it became punctured by the spherical probe. Chapatti textural properties such as elasticity and extensibility were able to be assessed. The distance to peak force ranged from 19.17 to 23.22 mm, with the greater the distance the more extensible the chapatti. Indian wheat variety *K 9107* was determined to be the least extensible, and wheat variety *HI 1531* the most extensible, of the Indian wheat varieties evaluated. The remaining parameter analysed from the second extensibility test was the work required to puncture a chapatti. The greater the area under the curve to peak force (E2A12) the more work was required to puncture the chapatti. Indian wheat variety *WH 542* required the least amount of work, 5226.29 gs^{-1}, and wheat variety *C 306* the greatest amount of work, 7126.57 gs^{-1}, to puncture a whole chapatti; and indicated chapattis of *C 306* were tougher than chapattis of *WH 542*. Significant differences between the Indian wheat varieties for E2Dis and E2A12 were determined ($p<0.05$).

Characterisation of the chapatti quality of the Australian wheat varieties

The chapatti quality of the Australian wheat varieties was evaluated by measuring the same chapatti quality attributes that were assessed for the Indian wheat varieties. Appendix 1.30 summarises the predicted means for each of the chapatti quality traits measured, for all of the Australian wheat varieties evaluated. The percentage bake loss, puffed height and percentage surface area of the chapatti puffed were measured and predicted means for selected Australian wheat varieties are shown in Table 4.16.

Table 4.16. Predicted means for the chapatti quality traits of percentage bake loss, puffed height and percentage surface area puffed of selected Australian wheat varieties.

Wheat Variety	Grade	Trial	BL (%)	PH (mm)	SAP (%)
HI 1531	-	*Indian*	*18.5*	*55.3*	*100.0*
PBW 175	-	*Indian*	*16.1*	*50.6*	*99.2*
Yitpi	AH	Binnu	15.3	48.1	100.0
		Williams	15.0	51.4	97.2
Fang	APW	Binnu	15.5	50.0	100.0
		Williams	15.2	51.1	98.3
Gladius	APW	Binnu	14.1	43.3	100.0
		Williams	14.4	47.2	97.6
Bumper	ASW	Binnu	15.4	51.9	100.0
		Williams	15.2	51.1	99.0
Kennedy	FEED	Binnu	15.6	44.2	100.0
		Williams	14.5	46.1	98.6
Calingiri	ASWN	Binnu	14.2	47.5	97.6
		Williams	13.9	41.7	87.9

LEGEND - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed

The percentage bake loss was shown to range from 14.08 to 16.96% for the Australian hard wheat varieties grown in the Binnu trial; and from 14.14 to 15.38% for the Australian hard wheat varieties grown in the Williams trial. The percentage bake loss of the Australian soft noodle wheat varieties from the Binnu and the Williams trials were shown to be lower, 13.40 to 14.17% and 13.30 to 13.92%; respectively. The lower percentage bake loss was expected due to the lower water absorption of the Australian soft noodle wheat chapatti flours, also a consequence of lower damaged starch, in comparison to the Australian hard wheat varieties. The results for percentage bake loss were not found to be significantly different between the Australian wheat varieties in the Binnu and the Williams trials. There was however a significant difference ($p<0.001$) in percentage bake loss between the Australian wheat varieties and the Indian wheat varieties. Nonetheless, percentage bake loss does not appear to have great value as an indicator of chapatti quality, as differences between the Australian wheat varieties in all but one of the data sets, Williams Hard, were not found to be significant.

Puffed height was another chapatti quality trait measured and it was identified that wheat variety *Bumper* had the greatest puffed height from the Binnu trial, and *Correll* and *Yitpi* had the highest puffed height from the Williams trial; see Table 4.16. Furthermore, wheat varieties *Gladius* and *Kennedy*, from the Binnu and the Williams trials, respectively, had the lowest puffed height of 43.3 mm and 46.1 mm; as shown in Table 4.16. The Australian soft noodle wheat varieties were also evaluated and *Calingiri* was identified as having the lowest puffed height for both trials; also presented in Table 4.16. Puffed height is an important quality characteristic of chapatti because it is the action of puffing which creates two layers, a defining characteristic of the product. However, the differences in puffed height observed between wheat varieties and between data sets, were not determined to be significant. The measurement scale used to assess puffed height may have contributed to this finding, with measurement to smaller increments possibly required. Nevertheless, the complete and full puffing of chapatti is essential to make a quality end product, and all of the Australian wheat varieties evaluated demonstrated good puffing abilities, as they all puffed to an acceptable level.

Percentage surface area of the chapatti puffed (SAP) contributed to the assessment of the complete and full puffing of chapatti. Puffing of the chapatti across the entire surface area is necessary for the formation of two distinct layers, and is an important characterising trait. The SAP values ranged from 100.0 to 96.8% and from 99.3 to 93.4% for the Australian hard wheat varieties from the Binnu and the Williams trials; respectively. Furthermore, the Australian soft noodle wheat varieties from the Binnu and the Williams trials had SAP values ranging from 100.0 to 96.9% and 96.5 to 87.9%; respectively. The data collected showed that the wheat varieties from the Binnu trial overall had higher percentage surface area values for puffing than the wheat varieties from the Williams trial. The difference observed between the two trials was found to be significant ($p<0.001$). Further analysis of the data determined that there were significant differences ($p<0.05$) for SAP values between the Australian soft noodle wheat varieties for both trials; however there were no significant differences between the Australian hard wheat varieties.

Colour of chapatti is another important quality characteristic because it contributes to end product appearance and needs to be acceptable and attractive to consumers. The colour of the raw chapatti dough sheets were measured on both side one and two; and the results for selected Australian wheat varieties are shown in Table 4.17. *EGABonnie Rock*, a hard wheat variety from the Binnu trial, was identified as having the lightest, higher *CIE L** values; and *Zippy* as having the darkest, lower *CIE L** values, raw chapatti dough sheets on sides one and two. In regards to redness, *EGA Wentworth* was determined to have raw chapatti dough sheets with the greatest redness, higher *CIE a** values, on both sides; and *Gladius* had the lowest redness of the hard wheat varieties from the Binnu trial tested. The raw chapatti dough sheets with the greatest yellowness, higher *CIE b** values, was *Espada*, and the lowest was *Tammarin Rock* and *Zippy*. The hard wheat varieties from the Williams trial, *Fang* and *Peake* had the highest *CIE L** values; and *Bumper* had the lowest, as described in Table 4.17. *Carnamah* had the highest and *Yipti* had the lowest *CIE a** values, see Table 4.17; and *Magenta* had the highest *CIE b** values and *Tammarin Rock* the lowest values. The varied findings between the hard wheat varieties from the two trials indicated factors other than genotype such as the amount of bran in the chapatti flours was affecting the colour of the raw chapatti dough sheets. Ash content was found to be significantly and positively correlated with raw and baked chapatti colour measurements ($p<0.05$).

Table 4.17. Predicted means for raw chapatti dough sheet colour measurements of selected Australian wheat varieties.

Wheat Variety	Grade	Trial	R1L (*L**)	R1a (*a**)	R1b (*b**)	R2L (*L**)	R2a (*a**)	R2b (*b**)
HI 1531	-	*Indian*	*72.81*	*4.70*	*24.07*	*72.64*	*4.73*	*24.27*
PBW 175	-	*Indian*	*70.49*	*5.56*	*24.51*	*70.28*	*5.65*	*24.86*
Yitpi	AH	Binnu	71.31	5.46	25.84	71.03	5.61	26.17
		Williams	71.64	5.32	25.78	71.48	5.47	25.95
Fang	APW	Binnu	71.51	5.69	25.35	71.48	5.74	25.46
		Williams	72.09	5.63	25.05	71.88	5.73	25.77
Gladius	APW	Binnu	72.43	4.83	25.48	72.07	5.03	26.08
		Williams	71.82	5.47	25.66	71.74	5.63	25.93
Bumper	ASW	Binnu	70.88	5.68	23.47	70.62	5.81	23.93
		Williams	68.80	6.52	24.14	68.82	6.51	24.13
Kennedy	FEED	Binnu	71.80	5.09	24.44	71.50	5.22	25.18
		Williams	70.40	6.38	25.54	70.44	6.32	25.60
Calingiri	ASWN	Binnu	73.81	4.57	23.35	73.67	4.61	23.87
		Williams	74.67	4.40	22.95	74.50	4.52	23.32

LEGEND - **R1L**: raw chapatti dough sheet colour *CIE L** of side one; **R1a**: raw chapatti dough sheet colour *CIE a** of side one; **R1b**: raw chapatti dough sheet colour *CIE b** of side one; **R2L**: raw chapatti dough sheet colour *CIE L** of side two; **R2a**: raw chapatti dough sheet colour *CIE a** of side two; **R2b**: raw chapatti dough sheet colour *CIE b** of side two

The colour of the raw chapatti dough sheets, of the Australian soft noodle wheat varieties in this research, were observed to be overall lighter in colour, higher *CIE L** values, and less red, lower *CIE a** values, in comparison to the Australian hard wheat varieties. A lower amount of bran in the chapatti flours of the Australian soft noodle wheat varieties would be the main reason for this observation. Differences in grain hardness resulted in the hard and soft wheat grains to mill differently; and consequently more bran, which is browner in colour, was retained in the hard wheat chapatti flours than in the soft noodle wheat chapatti flours (Pasha, Anjum, and Morris 2010). The soft noodle wheat variety from the Binnu trial which produced the lightest, *CIE L**, raw chapatti dough sheets was *Yandanooka*, and *Arrino* made the darkest raw chapatti dough sheets. The *CIE a** values, indicating redness, were determined to be similar for the wheat varieties *Arrino*, *Calingiri* and *Fortune*, and also similar for *Binnu* and *Yandanooka*; the last two wheat varieties having lower

*CIE a** values. *Fortune* had the greatest yellowness, *CIE b**, of the raw chapatti dough sheets, and *Arrino* and *Calingiri* had the lowest yellowness; see Table 4.17. Although not commonly reported for Indian wheat varieties, greater yellowness or golden colour is preferred by some consumers of chapatti (Gill, Sodhi, and Kaur 2005). In addition, *Fortune* had the lightest and *Arrino* had the darkest raw chapatti dough sheets on both sides, of the soft noodles wheat varieties from the Williams trial. *Arrino* had the greatest redness and *Fortune* the lowest redness of the raw chapatti dough sheets from the Williams trial. Lastly, the raw chapatti dough sheets with the greatest yellowness were from *Fortune*, and the lowest yellowness values were from *Calingiri*; as described in Table 4.17. Differences in colour of the raw chapatti dough sheets between the Australian hard and soft noodle wheat varieties were possibly due to differences in bran to endosperm ratios. The soft noodle wheat varieties had lower bran to endosperm ratios and this has been shown to affect flour and dough colour in this research.

Analysis of the raw chapatti dough sheet colour results, between the Binnu and the Williams trials, determined that there was a significant difference ($p<0.05$) between the *CIE a** values for both side one and side two. The differences seen in *CIE a** values may be due to differences in flour and dough composition affecting colour measurements. For example, differences in bran content, water absorption, particle size index, or level of enzymatic activity causing browning. Conversely, a comparison between the Indian and the Australian raw chapatti dough sheet colour values identified that *CIE L** and *b** values, lightness and yellowness were significantly different ($p<0.05$). The raw chapatti dough sheet colour of the Australian wheat varieties was shown to be lighter and yellower than the Indian wheat varieties. Dough darkening has negative implications on chapatti quality and the Australian wheat varieties may have good colour qualities to make chapatti which are desired to be creamy light brown to yellow in colour.

The colour of the baked chapatti is therefore of importance as it needs to meet acceptable colour requirements to be appealing and therefore of satisfactory quality. The baked chapatti colour was also measured on side one and two, as each side was baked for a different amount of time, as per the traditional method. Consequently, the appearance of the chapatti on each side was different. Table 4.18 presents the percentage change in colour for *CIE L* a** and *b** values for side one and side two of selected Australian wheat varieties. Predicted means for the baked chapatti colour measurements and the percentage change in chapatti colour for all Australian wheat varieties evaluated can be referred to in Appendix 1.29.

Table 4.18. Predicted means for the percentage change in chapatti colour, from raw to baked, of selected Australian wheat varieties.

Wheat Variety	Grade	Trial	%Δ1L*	%Δ1a*	%Δ1b*	%Δ2L*	%Δ2a*	%Δ2b*
HI 1531	-	*Indian*	*1.36*	*17.69*	*1.79*	*1.98*	*11.61*	*3.13*
PBW 175	-	*Indian*	*-0.07*	*14.68*	*4.37*	*1.21*	*7.90*	*3.94*
Yitpi	AH	Binnu	1.18	11.68	3.25	0.97	13.18	6.38
		Williams	-0.08	18.88	5.74	1.57	11.94	6.90
Fang	APW	Binnu	2.18	11.37	3.00	3.62	6.11	3.73
		Williams	1.14	12.98	3.23	2.03	10.18	4.42
Gladius	APW	Binnu	2.75	9.23	2.90	2.48	13.43	8.47
		Williams	2.87	10.69	4.33	2.89	12.85	6.63
Bumper	ASW	Binnu	2.51	7.96	0.47	2.87	6.90	4.14
		Williams	1.67	13.23	4.06	2.35	11.18	4.56
Kennedy	FEED	Binnu	2.21	8.66	-1.55	1.15	14.99	6.75
		Williams	2.97	11.94	3.76	1.68	15.13	5.86
Calingiri	ASWN	Binnu	2.98	10.11	0.64	2.96	10.81	6.62
		Williams	2.64	14.99	3.57	1.83	21.22	7.76

LEGEND - **%Δ1L***: percentage change in chapatti colour from raw to baked of *CIE L** of side one; **%Δ1a***: percentage change in chapatti colour from raw to baked of *CIE a** of side one; **%Δ1b***: percentage change in chapatti colour from raw to baked of *CIE b** of side one; **%Δ2L***: percentage change in chapatti colour from raw to baked of *CIE L** of side two; **%Δ2a***: percentage change in chapatti colour from raw to baked of *CIE a** of side two; **%Δ2b***: percentage change in chapatti colour from raw to baked of *CIE b** of side two

The percentage change in *CIE L** of the Australian wheat varieties evaluated from both trials ranged from -0.08 to 3.91%, and included side one and two measurements. Examples of the percentage change in *CIE L** values for selected wheat varieties are shown in Table 4.18. Overall there was a decrease in lightness of the chapatti after baking; therefore the chapatti became darker in colour. The range for the percentage change in *CIE L** values was not large, however the results were determined to be significant between the wheat varieties within each data set; Binnu Hard ($p<0.001$), Williams Hard ($p<0.001$), Binnu Soft ($p<0.05$) and Williams Soft ($p<0.001$). A comparison of the percentage change in *CIE L** between the Binnu and the Williams trials however was not significant. The findings demonstrated that end product colour differences were largely an effect of differences in genotype, rather than the processing method and environmental factors. In addition, there was a significant difference ($p<0.001$) between the percentage change in *CIE L** values of the Indian and the Australian wheat varieties. The results showed the Australian wheat varieties had a greater decrease in lightness after baking than the Indian wheat varieties.

The percentage change in *CIE a** values, Table 4.18, indicated a decrease in *CIE a**; based on the formula of raw minus baked colour value divided by raw colour value multiplied by 100. The decrease in *CIE a** values represented a decrease in the redness of the chapatti samples after baking. It was observed that the baked chapattis were not as brown in colour as the raw chapatti dough sheets. The percentage change in *CIE a** ranged from 5.95 to 22.71% for all of the Australian wheat varieties assessed and these results are reflected in Table 4.18. The percentage change in *CIE b** however was not as consistent and varied for different wheat varieties. The percentage change in *CIE b** was determined to range from -2.97 to 8.82% for the wheat varieties from the Binnu trial, and from 1.64 to 11.01% for the wheat varieties from the Williams trial. Nonetheless, for the majority of the Australian wheat varieties, the *CIE b** value decreased after baking, indicating a decrease in yellowness of the baked chapatti in comparison to the raw chapatti dough sheet. As earlier described, the intensity of brownness of the chapattis was generally observed to reduce after baking, which correlated to the measured decrease in redness and yellowness.

The percentage change in *CIE a** and *b** between wheat varieties within the Binnu Hard ($p<0.001$), Williams Hard ($p<0.05$), and Williams Soft ($p<0.05$) data sets were determined to be significant; except for the percentage change in *CIE a** of side one for the Williams Hard data set. Analysis of the differences between wheat varieties in the Binnu trial and the Williams trial identified significant differences ($p<0.001$) between the two trials for the percentage change in *CIE a** and *b** for side one. As side one of the chapatti had a longer baking time this may have allowed for greater changes in chapatti colour to take place.

Overall it was determined that the colour of the baked chapattis had generally lower *CIE L* a** and *b** values in comparison to the raw chapatti dough sheets. The Indian wheat varieties studied also found a similar trend; and this finding was further confirmed by Hatcher, Kruger and Dhaliwal (1997). The colour of the baked chapattis was not as light or as brown in colour, however a creamy light brown to slightly golden colour was observed. The percentage change in colour was largely found to show significant differences between the Australian wheat varieties in each data set, except for the Binnu Soft wheat varieties. The finding was not unexpected, as grain and flour colour are predominantly genetically determined traits (Morris 2002). In comparison, to the Indian wheat varieties, the percentage change in colour of the Australian wheat varieties was significant for *CIE L** side one and two ($p<0.001$), and *CIE a** and *b** side two ($p<0.05$).

Textural attributes of the chapattis were also evaluated to assess softness, tearing ability, and pliability characteristics, as they are important for eating quality (Srivastava, Prasada Rao, and Haridas Rao 2003). Two types of extensibility tests were performed to measure these textural attributes and gain better understanding of good and poor chapatti textural qualities of the Australian wheat varieties. The first extensibility test (E1) involved tearing a strip of chapatti of set dimensions. Three measurements from this test were used to assess textural properties; peak force to tear (E1PF), distance the chapatti strip extended until tearing (E1Dis), and the work required to tear the chapatti strip (E1A12). All of these factors relate to chapatti texture, such as softness and toughness from peak force values, which can then be related to eating quality of chapattis (Hemalatha et al. 2007; Dhaliwal et al. 1996). The second extensibility test measured similar attributes however in relation to the peak force (E2PF), distance (E2Dis) and work (E2A12) required to puncture a whole chapatti with a spherical probe. Predicted means for the texture measurements from these two tests, for selected Australian wheat varieties, are shown in Table 4.19; the complete data is presented in Appendix 1.29.

Table 4.19. Predicted means for texture measurements taken from two extensibility tests for selected Australian wheat varieties.

Wheat Variety	Grade	Trial	E1PF (g)	E1Dis (mm)	E1A12 (g.s)	E2PF (g)	E2Dis (mm)	E2A12 (g.s)
HI 1531	-	*Indian*	*587.76*	*5.36*	*1969.22*	*814.13*	*23.22*	*5854.76*
PBW 175	-	*Indian*	*484.18*	*5.83*	*1936.48*	*800.93*	*21.37*	*6231.90*
Yitpi	AH	Binnu	708.48	5.09	2475.20	918.58	23.09	6844.00
		Williams	696.32	5.13	2630.79	1005.86	22.02	7154.16
Fang	APW	Binnu	673.44	5.13	2405.60	932.74	23.07	6309.89
		Williams	615.14	5.26	2470.02	872.63	22.05	6342.23
Gladius	APW	Binnu	741.08	6.19	3252.07	1030.64	24.85	7341.44
		Williams	670.55	4.74	2340.16	819.11	20.02	6059.74
Bumper	ASW	Binnu	617.79	4.85	2080.22	900.97	21.15	6742.78
		Williams	674.54	5.82	2969.02	1045.78	21.23	8158.88
Kennedy	FEED	Binnu	653.71	6.44	2942.05	1089.76	24.00	8914.44
		Williams	694.35	5.78	2958.08	1073.89	22.66	8655.84
Calingiri	ASWN	Binnu	622.07	3.99	1801.21	854.53	21.40	6266.56
		Williams	565.90	4.69	2081.23	852.76	19.27	6384.72

LEGEND - **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti

Bumper a hard wheat variety from the Binnu trial was identified as requiring the least amount of force to tear and puncture chapatti samples in both extensibility tests; see Table 4.19. Furthermore, hard wheat variety *Magenta* from the Binnu trial required the least amount of work to tear and puncture chapatti samples, of the Australian wheat varieties evaluated. The findings indicated the chapatti samples of *Bumper* and *Magenta* were the least tough, and thus had softer texture chapattis which were of good quality, but also had different extension properties. Furthermore, *Espada* required the least amount of work to tear and puncture chapatti samples; and chapatti samples of *Zippy* extended the greatest distance in both extensibility tests, of the hard wheat varieties in the Williams trial. Lower values for peak force indicated softer texture chapatti which was of good quality; the lower amount of work required was also generally an indication of softer texture but could also represent limited stretching capabilities. Lastly, extensibility values which were not too low, but not too high were considered to represent good quality chapatti. Low extensibility values can represent chapatti which is brittle and not pliable, whereas very high extensibility values can indicate elastic chapatti which is hard to tear, and also not desirable. For the Australian soft noodle wheat varieties, *Arrino*, from the Binnu trial, needed the greatest amount of force to puncture and tear chapatti samples which indicated that it made the toughest and poor quality chapattis. Whereas, *Calingiri* was noted to extend the least in both extensibility tests; the results are shown in Table 4.19, and therefore was not very elastic. Lastly, *Fortune*, the soft noodle wheat variety from the Williams trial, was identified as requiring the least amount of work, and also extended the shortest distance, in both extensibility tests; and this may indicate a soft texture chapatti.

Differences between the Australian wheat varieties for the different texture measurements were mostly significant for the hard wheat varieties ($p<0.05$) but not significant between the soft noodle wheat varieties. Chapatti texture is firstly affected by protein and then starch properties, over storage time from starch retrogradation (Shaikh, Ghodke, and Ananthanarayan 2007). The chapattis were assessed fresh, thus the findings indicated there was greater variation in protein content and quality between the hard wheat varieties than the soft noodle wheat varieties evaluated, and this would be expected. Overall the range in results for the textural attributes of the wheat varieties in the Binnu and the Williams trials were observed to be similar; and this was further confirmed with only E2Dis being significantly different ($p<0.001$) between the two trials. Finally, in comparison to the Indian wheat varieties the results from the second extensibility test (E2), which involved puncturing a whole chapatti, were determined to be significantly different ($p<0.05$) to the Australian wheat varieties. The initial findings suggested that some or most of the Australian wheat varieties did not produce chapatti texture to the standard of the Indian wheat varieties.

Comparison of the chapatti quality of the Indian and the Australian wheat varieties

The chapatti quality of the Indian and the Australian wheat varieties was compared using the chapatti quality traits measured and principle component analysis (PCA) was conducted. The following chapatti quality traits were included in the analysis;

percentage bake loss, puffed height, percentage surface area of the chapatti puffed, baked chapatti colour measurements for side one and two, and the texture measurements from the two extensibility tests performed. The PCA output with latent vector loadings for this analysis is contained in Appendix 1.31. Figure 4.7 displays the biplot of the PCA of these chapatti quality traits for the Indian and the Australian wheat varieties assessed. A convex hull has been drawn around the wheat varieties in each data set on the biplot; Binnu Hard are represented as black, Williams Hard in blue, Indian as green, Binnu Soft as red, and Williams Soft in light blue.

The first principal component (PC-1) was shown to determine 36.52% of the variation between the wheat varieties. The quality traits of baked chapatti colour *CIE L** and *a**, representing lightness and redness, and the distance and work required to tear a chapatti strip, were identified as being important. Figure 4.7 shows the vectors representing baked colour *CIE L** (B1L and B2L) and *a** (B1a and B2a) which were the main drivers separating the hard and the soft noodle wheat varieties. The Australian soft noodle wheat varieties are shown to be grouped on the left hand side of the biplot, and the Indian and the Australian hard wheat varieties are clustered on the right hand side. The textural measurements of distance (E1Dis) and work required (E1A12) to tear a chapatti strip, were also important quality traits determining differences between the wheat varieties. The vectors for these traits can been seen to travel from the bottom left of the biplot to the top right, see Figure 4.7, and the wheat varieties are shown to be separated along these vectors.

The second principle component (PC-2) explained 17.91% of the variation in chapatti quality between wheat varieties, and it was determined that chapatti textural traits were largely determining differences. The work required to tear (E1A12), and puncture (E2A12) chapatti samples; and the peak force to puncture a chapatti (E2PF) were important quality traits. The baked chapatti colour *CIE b** (B2b), indicating yellowness, was also a key characteristic determining differences between wheat varieties. Figure 4.7 shows the vectors for these quality traits travel from the bottom left to the top right of the biplot for the textural attributes of importance, and from the top to the bottom of the biplot for the baked chapatti colour *CIE b**. Wheat varieties are shown to be separated along all of these vectors.

Figure 4.7. Biplot of PCA of chapatti quality traits for the Indian and the Australian wheat varieties.

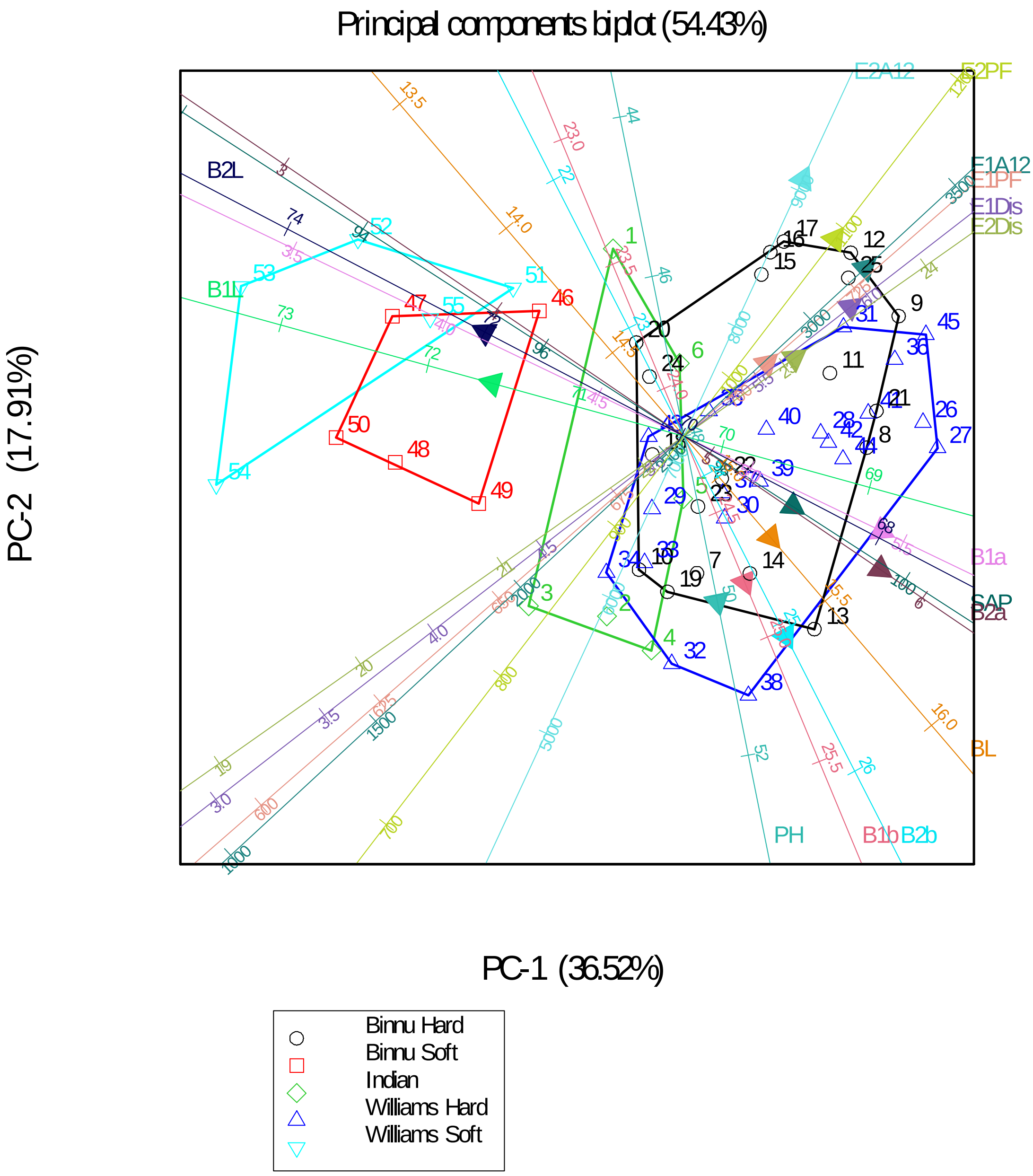
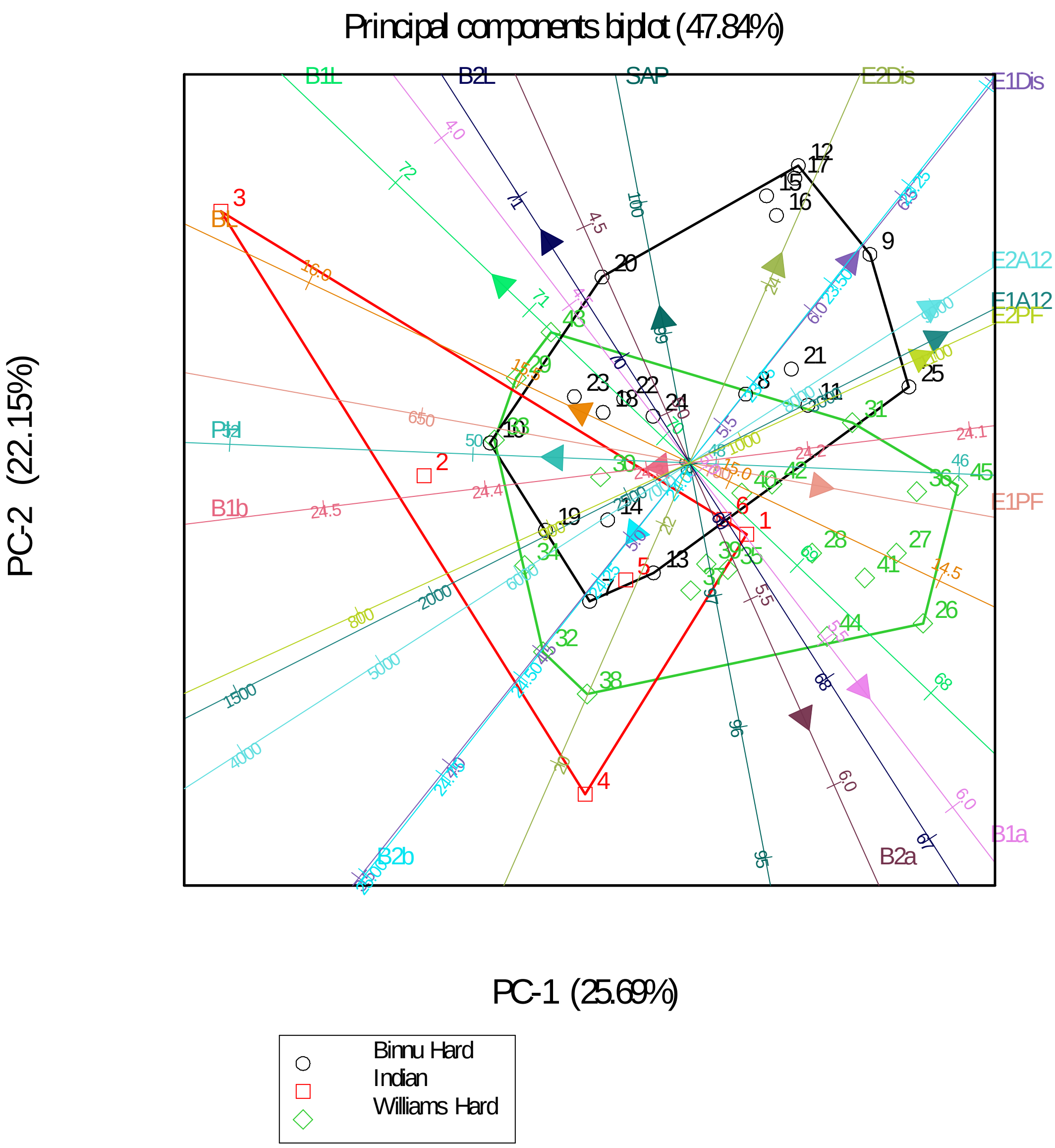


Figure 4.7 shows the similarity in chapatti quality of the wheat varieties in the Binnu and the Williams trials, observed from the overlap of these two data sets with each other. Furthermore, some of the Australian hard wheat varieties (black and blue) were shown to have similar chapatti quality as some of the Indian wheat varieties

(green), see Figure 4.7; there are wheat varieties from these data sets positioned near each other. The inclusion of the Australian soft noodle wheat varieties in the PCA however highlighted their differences in chapatti quality to the Indian and the Australian hard wheat varieties. Therefore PCA was performed using the chapatti quality trait data of the Indian and the Australian hard wheat varieties only and a biplot, Figure 4.8, was generated. The PCA output containing the latent vector loadings can be referred to in Appendix 1.32.

Figure 4.8. Biplot of PCA of chapatti quality traits for the Indian and the Australian hard wheat varieties.



The first principle component (PC-1) was shown to explain 25.69% of the variability in chapatti quality between the hard wheat varieties. The quality traits identified as being important for determining differences were; the work required to tear (E1A12) and puncture (E2A12) the chapatti samples, the peak force to puncture a chapatti (E2PF) and the baked chapatti colour *CIE L** for side one (B1L). The vectors for the textural traits of importance can be seen in Figure 4.8 to travel from the left to the right of the biplot; and the *CIE L** chapatti colour for side one from the right to the top left of the biplot. Wheat varieties are separated on the biplot by these vectors, and the Australian hard wheat varieties were shown to have higher values for the textural attributes highlighted as being important, indicating tougher stretchier chapatti, in comparison to the Indian wheat varieties.

The second principle component (PC-2) accounted for 22.15% of the variation and was driven by the traits of baked chapatti colour *CIE L** side two (B2L) and *CIE a** colour on side one and two (B1a and B2a). The distance to tear (E1Dis) and the distance to puncture (E2Dis) chapatti samples, or the extensibility measurements, were also important traits. The vectors for these traits are displayed going from the top to the bottom (B1a, B2a), and from the bottom to the top of the biplot (B2L, E1Dis, E2Dis); see Figure 4.8. Differences in chapatti texture of the Australian hard wheat varieties and the Indian wheat varieties were also main distinguishing factors. The Australian hard wheat varieties were shown to have higher values for extensibility, indicating the chapattis were able to be stretched further and were more elastic. In contrast, the two Indian wheat varieties, *HI 1531* and *PBW 175,* with good chapatti quality, were shown to have softer texture, lighter baked colour, higher bake loss and higher puffed height.

Further observations from the biplot, Figure 4.8, revealed that the Indian wheat varieties each had different chapatti quality; apart from wheat varieties *C 306* (1) and *PBW 343* (6), which were shown to be similar. Furthermore, some of the Australian hard wheat varieties were observed to have similarities in chapatti quality to the Indian wheat varieties. The Australian hard wheat varieties that were shown to be clustered near the Indian wheat varieties in the biplot were considered to have similar chapatti quality and are described in Table 4.20.

Table 4.20. Australian hard wheat varieties with similar chapatti quality to the Indian wheat varieties.

Indian wheat variety	Australian hard wheat variety from the Binnu trial	Australian hard wheat variety from the Williams trial
C 306 (1) PBW 343 (6)	None	Cascades (28) Guardian (35) Peake (39) Tammarin Rock (40) Wyalkatchem (42)
K 9107 (5)	Bumper (7) Espada (13)	Gladius (34) Mace (37)
WH 542 (4)	None	Espada (32) Magenta (38)
PBW 175 (2)	Correll (10)	Fang (33)
HI 1531 (3)	None	None

LEGEND - The number in brackets () is the number of the associated wheat variety located on Figure 4.8.

Quality traits determining differences in chapatti quality

The process of understanding the chapatti quality traits which were important in determining differences between the wheat varieties evaluated, involved further PCA on each data set. Table 4.21 displays the latent vectors and their loadings for the first principle component (PC-1) generated from each of these analyses. Appendix 1.33 contains the complete PCA output of the latent vector loadings. The chapatti quality traits highlighted in blue, in Table 4.21, were recognised as being important for determining quality differences between wheat varieties in each of the data sets; Indian, Binnu Hard, Williams Hard, Binnu Soft and Williams Soft. The results showed that the chapatti quality traits which contributed to quality differences in PC-1 were not consistent across each of the data sets. Nevertheless, several key chapatti quality traits were identified as driving differences in more than one data set. The chapatti quality traits included puffed height; chapatti colour *CIE L** and *a** side one; the distance and work required to tear a chapatti strip; and the peak force and work required to puncture a chapatti. These chapatti quality traits were also shown to be important in the previous PCA analyses; see Figures 4.7 and 4.8. Therefore, higher puffed height, higher *CIE L** values, lighter, lower *CIE a** values, less red, and the lower the distance and work to tear, the better quality chapatti.

In addition, the first principle component was shown to account for 50.30% of the variability between the Indian wheat varieties. The chapatti quality traits of puffed height, percentage surface area puffed, chapatti colour *CIE L** side one, and the peak force and work required to tear a chapatti strip were identified as being important; see Table 4.21. The PCA results for the Indian wheat varieties further confirmed the importance of puffing, colour and texture measurements needed to describe chapatti quality.

Table 4.21. Table of the first principle component (PC-1) of PCA correlation matrix analysis for chapatti quality traits.

Trait or Latent vectors[1]	PC-1 Loadings				
	Indian	Binnu Hard	Williams Hard	Binnu Soft	Williams Soft
BL	0.216	-0.127	-0.024	-0.080	-0.241
PH	0.318	-0.267	-0.054	-0.327	0.303
SAP	0.312	0.171	0.057	0.186	0.294
B1L	0.356	0.234	-0.334	-0.384	-0.217
B1a	-0.224	-0.266	0.318	0.378	0.257
B1b	0.185	-0.091	-0.284	0.125	-0.050
B2L	0.285	0.293	-0.295	-0.371	-0.276
B2a	-0.155	-0.322	0.270	0.398	0.298
B2b	0.212	-0.179	-0.237	0.201	0.043
E1PF	-0.306	0.140	-0.076	0.240	0.066
E1Dis	0.215	0.337	0.342	0.038	0.245
E1A12	-0.299	0.342	0.318	0.144	0.325
E2PF	-0.239	0.359	0.264	0.026	0.343
E2Dis	0.276	0.160	0.278	-0.336	0.299
E2A12	-0.178	0.351	0.336	-0.122	0.324
Number of wheat varieties	**6**	**19**	**20**	**5**	**5**
Principal component 1 (%)	**50.30**	**34.13**	**36.35**	**35.17**	**52.60**

[1]**LEGEND** - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed; **B1L**: baked chapatti colour *CIE L** of side one; **B1a**: baked chapatti colour *CIE a** of side one; **B1b**: baked chapatti colour *CIE b** of side one; **B2L**: baked chapatti colour *CIE L** of side two; **B2a**: baked chapatti colour *CIE a** of side two; **B2b**: baked chapatti colour *CIE b** of side two; **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti

Relationships between flour, chapatti, and flour and chapatti quality traits of the Indian and the Australian hard wheat varieties

Correlations were performed to investigate relationships between flour, between chapatti, and between flour and chapatti quality traits. The Indian, Binnu Hard and Williams Hard data sets were analysed individually. Correlations between the Australian soft noodle wheat varieties were not reported due to their significantly different flour and chapatti quality. Appendix 1.34 contains the correlations for each of the data sets discussed; significant positive (red) and significant negative (green) correlations ($p<0.05$) have been highlighted.

Correlations between flour quality traits

Significant correlations ($p<0.05$) between flour quality traits in the Indian, Binnu Hard and Williams Hard data sets are shown in Table 4.22. The flour quality traits showed expected significant correlations between measurements which were a function of each other. For example, dough development time was positively correlated to dough stability and time to breakdown, and negatively correlated to mixing tolerance index. These four dough properties were measurements taken from the farinograph test, and they were shown to be significantly correlated in each of the data sets. Furthermore, significant correlations were generally observed for quality traits which were measuring similar attributes such as dough properties or starch quality. For example, dough measurements such as resistance to extension measured from the extensigraph, was significantly and positively correlated to dough development time, stability and time to breakdown measured on the farinograph. In addition, the starch pasting properties measured from the RVA, flour swelling volume and falling number were also found to have significant correlations; see Table 4.22. The flour quality traits characterised and their relationships with protein and starch qualities were expected as the different quality tests, which mainly measure protein and starch properties, generally measure similar attributes but in different ways. Overall, there were some similarities and differences in the significant correlations identified between flour quality traits in each of the data sets analysed.

Table 4.22. Significant relationships (p<0.05) between flour quality traits in the Indian, Binnu Hard and Williams Hard data sets.

Quality Trait[1]	INDIAN Positive correlation	BINNU HARD Positive correlation	WILLIAMS HARD Positive correlation	INDIAN Negative correlation	BINNU HARD Negative correlation	WILLIAMS HARD Negative correlation
Ash	Bkd, FSV	FSb, Fa, Fb, PSI0.1, PSI0.5, SetB	PTime		BkD, Ext, FL, FSV	
BkD	FSV, FSa, PV	FSV, PV	FL, FSV, PV	DSAtoF, DSDisF, FSL	DT, Fb, SetB, TtoB	PSI0.1, PSI0.5, PSI0.9, SetB
DS	WA	WA			PSI0.9	DSAtoF, DSDisF, Ext, WP
DSAtoF	DSDisF, DSF, FL, FSL, WA	DSDisF, DSF	DSDisF, DSF, Ext, WP	FSV	SetB	DT, Rmax
DSDisF	DSF, FL, FSL	DSF	DSF, Ext, WP	FSV, FSa	SetB	Rmax
DSF	FL, FSL, FSb, WA	MTI	Ext, WP		SetB	DT, Rmax
DT	PTime, PV, RMax, Stab, TtoB	Rmax, SetB, Stab, TtoB	FV, Rmax, SetB, Stab, TtoB	Fb, MTI	FSV, MTI, PV	FSV, MTI
Ext	WP	FL,	WP		FSa, FV, Fa, PTemp, SetB	FV, PV, Trgh
FL	FN, FSb, Trgh, WA, WP	FSL, FSV, PTime	FSL, FSV	PTemp	FN, FSa, FSb, Fa, Fb, PSI0.1, PSI0.5, PSI0.9, SetB	FSa, Fa, PSI0.1, PSI0.5, PSI0.9, WA
FN	Trgh, WA, WP	FSb, FV, Fb, PSI0.5, PSI0.9, Trgh	FV, PTime, PV, Rmax, SetB, Stab, Trgh, TtoB	PTemp		MTI, PSI0.9, PTemp
FSL		PTime		FSV, FSa	FSa, Fa,	FSa, Fa,

					PSI0.5, WA	PSI0.1, WA
FSV	FSa	PTime, PV	PV		FSb, Fa, Fb, PSI0.1, SetB, TtoB	PSI0.1, PSI0.5, PSI0.9, SetB
FSa	Fa, PTime, PV	Fa, WA	Fa, WA	PSI0.5		Fb
FSb		Fb, PSI0.1, PSI0.5, PSI0.9	FV, Fb, SetB	Rmax, TtoB		
FV	SetB, Trgh	SetB, Trgh	Fb, PTime, Rmax, SetB, Trgh,	Fb, MTI, PSI0.1		WA, WP
Fa	PTime	PSI0.1, PSI0.5, PSI0.9	PSI0.1, PSI0.9, WA		PTime	
Fb	MTI,PSI0.1	PSI0.1, PSI0.5, PSI0.9, SetB	SetB	PTime, PV, Rmax, SetB, Stab, Trgh, TtoB	PTime	
MTI				Rmax, TtoB	Rmax, SetB, Stab, TtoB	Rmax, Stab, TtoB
PSI0.1	PSI0.5, PTemp	PSI0.5, PSI0.9	PSI0.5, PSI0.9	PV, Trgh	PTime	PTime, PV
PSI0.5	PTemp	PSI0.9	PSI0.9	PV, Trgh	PTime	PTime, PV
PSI0.9	Rmax, SetB				PTime	PTime, PV
PTemp				Trgh, WP		PTime
PTime	PV, Stab, TtoB		PV, Trgh			
PV	Stab, Trgh	Trgh	Trgh		SetB, TtoB	
Rmax	SetB, TtoB	SetB, Stab, TtoB	SetB, Stab, TtoB			
SetB		TtoB				
Stab	TtoB	TtoB	TtoB			
Trgh	WP					WP
WA			WP			

[1]**LEGEND** - **Ash**: ash content; **Bkd**: starch pasting breakdown; **DS**: damaged starch content; **DSAtoF**: dough stickiness area under the curve to peak force; **DSDisF**: dough stickiness distance to peak force; **DSF**: dough stickiness peak force; **DT**: dough development time; **Ext**: dough extension; **FL**: flour colour *CIE L**; **FN**: falling number; **FSL**: flour slurry colour *CIE L**; **FSV**: flour

swelling volume; **FSa**: flour slurry colour *CIE a**; **FSb**: flour slurry colour *CIE b**; **FV**: starch pasting final viscosity; **Fa**: flour colour *CIE a**; **Fb**: flour colour *CIE b**; **MTI**: farinograph mixing tolerance index; **PSI0.1**: 10th percentile of the particle size distribution; **PSI0.5**: 50th percentile of the particle size distribution; **PSI0.9**: 90th percentile of the particle size distribution; **PTemp**: starch pasting temperature; **PTime**: starch pasting peak time; **PV**: starch pasting peak viscosity; **Rmax**: dough maximum resistance to extension; **SetB**: starch pasting setback; **Stab**: farinograph dough stability; **Trgh**: starch pasting trough; **TtoB**: farinograph dough time to breakdown; **WA**: farinograph water absorption; **WP**: protein content.

The relationships between flour quality traits which were important for determining differences between the Indian and the Australian wheat varieties were investigated. Significant and positive correlations were found between damaged starch content and water absorption in the Indian and Binnu Hard data sets; and dough extension and protein content in the Indian and Williams Hard data sets. Significant negative correlations between flour colour *CIE L** and particle size index measurements; and significant positive correlations between flour colour *CIE a** and *b** and particle size index measurements, were particularly noted for the Australian wheat varieties.

Correlations between chapatti quality traits

The relationships observed between chapatti quality traits were largely between the related chapatti colour and texture measurements; see Table 4.23. Generally, the raw chapatti dough sheet colour and the baked chapatti colour measurements, for *CIE L* a** and *b**, were shown to be significantly correlated in all data sets; Indian, Binnu Hard and Williams Hard. There were also some significant correlations between baked chapatti colour and the extensibility test measurements; see Table 4.23. For example, in the Indian data set, as baked chapatti colour *CIE L** increased extensibility from the puncture test also increased but peak force and work required to tear a chapatti decreased. Also, in the Indian data set, as baked chapatti colour *CIE a** increased, the work required to tear and puncture chapatti also increased. Therefore, the Indian wheat varieties with better textural qualities had lighter and less red chapatti colour, which may be a result of selection for these characteristics by Indian breeders, but the relationship between texture and lightness needs further investigation. Furthermore, the extensibility test one and extensibility test two measurements, which measured textural attributes of chapatti by tearing and puncturing chapatti samples, were determined to be significantly correlated with each other; and this was expected as they were measuring similar textural properties. Lastly, significant and positive correlations between percentage bake loss and puffed height in the Binnu Hard and Williams Hard data sets; and puffed height and raw chapatti colour *CIE L** and *b**, and percentage surface area of the chapatti puffed were identified.

Table 4.23. Significant relationships (p<0.05) between chapatti quality traits in the Indian, Binnu Hard and Williams Hard data sets.

Quality Trait[1]	INDIAN Positive correlation	BINNU HARD Positive correlation	WILLIAMS HARD Positive correlation	INDIAN Negative correlation	BINNU HARD Negative correlation	WILLIAMS HARD Negative correlation
B1L	B2L, BL, E2Dis, P1a, P2L, PH, R1L, R2L, SAP	B2L, P1a, R1L, R2L	B2L, B2b, P1a, R1L, R2L, R2b	B1a, E1A12, E1PF	B1a, B2a, R1a, R2a	B1a, B2a, E1Dis, E2Dis, P1L, R1a, R2a
B1a	B2a, R1a, R2a	B2a, R1a, R2a	B2a, E1A12, E2A12, P1L, R1a, R2a	B2L, BL, E2Dis, R1L, R2L	B2L, R1L, R2L	B2L, P1a, P1b, PH, R1L, R2L
B1b	B2b, R1b, R2b	B2b, P1b, P2b, R1b, R2b	B2b, R1b, R2b			E1A12, E1Dis, E2A12, E2Dis
B2L	B2b, E2Dis, P2a, R1L, R2L	E2PF, P2a, P2b, R1L, R2L	R1L, R2L	B2L	B2a, BL, P2L, R1a, R2a	B2a, E1Dis, R1a, R2a
B2a	R1a, R2a	BL, PH, R1a, R2a	E1A12, R1a, R2a	BL, E2Dis, P1L, P2a, R1L, R2L	P2a, P2b, R1L, R2L	P2a, R1L, R2L
B2b	P1b, R1b, R2b, SAP	P1b, R1b, R2b	P1a, P1b, R1b, R2b	E2A12		P1L
BL	E2Dis, P2a, R1L, R2L	P2L, PH	PH	R1a, R2a	P2a, P2b	
E1A12	E1PF, E2PF	E1Dis, E1PF, E2A12, E2Dis, E2PF	E1Dis, E2A12, E2PF, R1a, R2a	P1a, P1b, PH, SAP	PH	

E1Dis		E2A12, E2Dis, E2PF, P1L, SAP	E2A12, E2Dis	E1PF	P1b, PH	E1PF, R1b, R2b
E1PF				P1b, PH, R2b, SAP		E2Dis, SAP
E2A12	E2PF	E2PF, SAP	E2Dis, E2PF, R1a, R2a	P1a	P1b	R1b, R2b
E2Dis	R1L, R2L		SAP	R1a, R2a		R1b, R2b
E2PF		R1L, R2L	R1a, R2a	P1a, P1b		
P1L	R1L, R2L	P2L, R1L, R2L	P2L	R1a, R2a	P1a, P1b	P1a, P1b, PH, R1b, R2b
P1a	PH, SAP	P1b, PH	P1b, PH, R1b, R2b			P2L
P1b	R1b, R2b, SAP	R1b, R2b	P2b, R1b, R2b			
P2L	R1L, R2L				P2a, P2b	P2a
P2a		P2b	P2b		PH	
P2b		R2b			PH	
PH	R1b, R2L, R2b, SAP	R1a				
R1L	R2L	R2L	R2L	R2a	R1a, R2a	R1a, R2a
R1a	R2a	R2a	R2a		R2L	R2L
R1b	R2b, SAP	R2b	R2b			
R2L					R2a	R2a
R2b	SAP					

[1]**LEGEND** - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed; **B1L**: baked chapatti colour *CIE L** of side one; **B1a**: baked chapatti colour *CIE a** of side one; **B1b**: baked chapatti colour *CIE b** of side one; **B2L**: baked chapatti colour *CIE L** of side two; **B2a**: baked chapatti colour *CIE a** of side two; **B2b**: baked chapatti colour *CIE b** of side two; **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti; **R1L**: raw chapatti dough sheet colour *CIE L** of side one; **R1a**: raw chapatti dough sheet colour *CIE a** of side one; **R1b**: raw chapatti dough sheet colour *CIE b** of side one; **R2L**: raw chapatti dough sheet colour *CIE L** of side two; **R2a**: raw chapatti dough sheet colour *CIE a** of side two; **R2b**: raw chapatti dough sheet colour *CIE b** of side two; **P1L**: percentage change in chapatti colour from raw to baked of *CIE L** of side one; **P1a**: percentage change in chapatti colour from raw to baked of *CIE a** of side one; **P1b**: percentage change in chapatti colour from raw to baked of *CIE b** of side one; **P2L**: percentage change in chapatti colour from raw to baked of *CIE L** of side two; **P2a**: percentage change in chapatti colour from raw to baked of *CIE a** of side two; **P2b**: percentage change in chapatti colour from raw to baked of *CIE b** of side two

Correlations between flour and chapatti quality traits

Relationships between flour and chapatti quality traits were determined and are described in Table 4.24. Significant positive correlations were found in all of the data sets for baked chapatti colour *CIE L** and *a** and flour and flour slurry, *CIE L** and *a**; respectively. For baked chapatti colour *CIE b**, significant positive correlations with ash content, flour and flour slurry *CIE b**, were identified in the Binnu Hard and Williams Hard data sets. In addition, flour slurry *CIE L** had a significant negative correlation with baked chapatti colour *CIE a** in all of the data sets; see Table 4.24.

Table 4.24. Significant relationships (p<0.05) between flour and chapatti quality traits in the Indian, Binnu Hard and Williams Hard data sets.

Quality Trait[1]	INDIAN Positive correlation	BINNU HARD Positive correlation	WILLIAMS HARD Positive correlation	INDIAN Negative correlation	BINNU HARD Negative correlation	WILLIAMS HARD Negative correlation
Ash	E2A12	B1b, B2a, B2b, P1b, R1a, R1b, R2a, R2b	B2b, R2b	P2L	E1A12, E1Dis, E2A12, E2PF, P1L	
B1L	DSFtoF, DSDisF, DSF, FL, FN, FSL, FSb, WA	FSL	FL, FSL, Fb			FSa, Fa, WA, WP
B1a	FSa, Fa, PTime	FN, FSa, Fa, WA	FSa, Fa, WA	DSFtoF, DSDisF, DSF, FSL	Ext, FL, FSL	FL, FSL, Fb
B1b	Ext	FN, FSb, Fb, PSI0.1, WP	FSb, FV, Fb, SetB		FL, FSV	
B2L	DSFtoF, DSDisF, DSF, FL, FSL	FL, FSL, PTime	FL, FSL	PSI0.9	FSa, Fa, PSI0.5	FSa, Fa, WA
B2a	FSa, Fa, PTime	FSa, Fa, PSI0.1, PSI0.5, PSI0.9, WA	FSa, Fa, WA	DSAtoF, DSDisF	Ext, FL, FSL, PTime	FL, FSL

B2b		FN, FSb, Fb, PSI0.1, PSI0.5, PSI0.9, WP	FSb, FV, Fb, SetB		FL, FSV, PTime	
BL	DSFtoF, DSDisF, DSF, FSL, WA	PSI0.1, PSI0.5, PSI0.9	MTI	FSa, Fa, PTime	FL, FSL, Rmax, TtoB	Stab, WP
BkD		E1Dis, E2A12, SAP	E1A12, E1Dis, E2A12, E2PF, P1a	P2L, R1L, R2L	P1b, R1b, R2b	
DS		SAP	P1a		E1PF	E2A12, E2Dis, E2PF, P1L
DSAtoF	P2L, R1L, R2L	E1A12, E1Dis, P1L	E2Dis	R2a	P1a, PH	P1b, P2b
DSDisF	E2Dis, P2a, R1L, R2L	E1A12, E1Dis, P1L	E2Dis	R2a	P1a, PH	P2b
DSF	E2Dis, P1a, P2L, PH, R1L, R2L	E1A12, E1Dis, P1L, P2L	E2Dis		P1a	E1PF, P1b
DT			E1PF	E1Dis, P1L, P2L, R1L, R2L	PH	
E1A12	PSI0.1, PTemp	Ext	FSV, FSA, PV, WA	FL, FN, Trgh, WA, WP	Fa, SetB	SetB
E1Dis	FSb	Ext, FL, FSV	FSV, FSa, WA	Rmax, SetB, TtoB	Fa, SetB	FSL, FV, Fb, SetB
E1PF	PTemp	FN	FV, Rmax, Trgh, TtoB	FL, FSb	WA	Ext
E2A12	PSI0.1	Ext, FL, FSV, PV	FSV, FSa, PV		SetB	Fb, SetB
E2Dis	WA	Ext	Ext, WA, WP	Fa, PTime	PTemp	FN, FV, Fb, Rmax, SetB, Trgh

E2PF		Ext, Stab	FSV, PV		PTemp	
Ext	PH, R1b, R2b				R1a, R2a	
FL	P1a, P2a, PH, R2L, SAP	P2a, R1L, R2L	R1L, R2L		P1b, PH, R1a, R1b, R2a, R2b	R1a, R2a
FN	P1a, PH, SAP	R1a, R1b, R2a, R2b	P2b, R1b, R2b			
FSL	P2a, R1L, R2L	P2b, R1L, R2L	R1L, R2L		R1a, R2a	R1a, R2a
FSV		SAP	P1a		P1b, R1b, R2b	
FSa	R1a, R2a	PH, R1a, R2a	R1a, R2a		P2b, R1L, R2L	P2b, R1L, R2L, R2b
FSb	P2L, R1L, R2L	P1b, R1b, R2b	P1a, P1b, R1b, R2b			P1L
FV			P2b, R1b, R2b			
Fa	R1a, R2a	PH, R1a, R2a	R1a, R2a			P1a, P1b, P2a, P2b, R1L, R1b, R2b
Fb	P1L	P1b, R1b, R2b	P1a, P1b, R1b, R2b			P1L
P1L				PSI0.9, PTime, PV, Rmax, TtoB		Trgh
P1a	WA		PV, Trgh	PSI0.1, PTemp		
P1b		PSI0.1, PSI0.5, PSI0.9, SetB	Trgh		PTime	
P2L			PSI0.1	Stab, TtoB		
P2a		PTime, Rmax, Stab	Stab, TtoB		PSI0.1, PSI0.5, PSI0.9	PSI0.1, PSI0.5, PSI0.9
P2b			WA	PSI0.9		

PH	Trgh, WP	PSI0.1, PSI0.5, PSI0.9		PTemp	PTime	
PSI0.1		R1a, R1b, R2b		SAP		
PSI0.5		R1a, R1b				
PSI0.9		R1a				
PTemp				SAP		
PTime	R1a, R2a	SAP		R1L	R1a	
PV	R1a	SAP				
R1L				Rmax, TtoB		WA
R1a			WA			
R1b		SetB, WP	SetB, Trgh			
R2L				Rmax		
R2a			WA			
R2b		WP	SetB, Trgh			
SAP	Trgh, WP	WA	WA		SetB	

[1]**LEGEND** – See Legends for Tables 4.22 and 4.23

For percentage bake loss, only water absorption was found to have a significant positive correlation in the Indian data set. Whereas, puffed height was identified as correlating to a number of flour quality traits in the Indian and the Binnu Hard data sets; although they were different flour quality traits in each. Puffed height had significant positive correlations with protein content, dough extension, dough stickiness force, flour colour *CIE L** and falling number in the Indian data set. On the other hand, puffed height had significant positive correlations with flour and flour slurry *CIE a**; and significant negative correlations with dough stickiness properties, dough development time, and flour colour *CIE L** in the Binnu Hard data set. Puffed height is a measurement of the action of chapatti puffing and would therefore be expected to be influenced by both starch and protein qualities of the flour and dough. Factors such as water absorption, as it is a steam leavened product; and protein strength, extensibility and elasticity were shown to affect the chapatti's ability to puff.

Percentage surface area of the chapatti puffed (SAP) was also determined to have significant positive correlations to flour quality traits; however they were different for each data set; see Table 4.24. The percentage surface area of the chapatti puffed had significant positive correlations with protein content, falling number and flour colour *CIE L** for the Indian data set. The Binnu Hard and Williams Hard data sets, had significant positive correlations between SAP and water absorption; and the Binnu Hard data set additionally had significant positive correlations between SAP and damaged starch content, flour swelling volume and peak viscosity. Similarly, to chapatti puffed height, both starch and protein qualities have been shown to have an effect on the ability of chapattis to puff completely across the surface.

In regards to the two extensibility tests, which measured force, work and distance to describe textural properties of chapatti, these textural attributes were shown to be significantly correlated to a wide range of flour quality traits in each of the data sets; see Table 4.24. The significant correlations that were identified in more than one data set are discussed. The distance a chapatti strip can be stretched until tearing was found to have a significant negative correlation to starch pasting setback in all of the data sets. Furthermore, there was a significant positive correlation to flour swelling volume in the Binnu Hard and Williams Hard data sets. Additionally, the work required to tear the chapatti strip was also shown to be significantly and negatively correlated to starch pasting setback in the Binnu Hard and Williams Hard data sets. A possible explanation for these relationships relates to the importance of both starch and protein qualities for chapatti textural quality. When the protein qualities in a flour and dough are more dominant than the starch qualities, then the effect on chapatti texture may be mainly associated with the dominant attributes. In addition, the distance the chapatti stretched by puncturing, measured in the second extensibility test, had a significant positive correlation to water absorption in the Indian and Williams Hard data sets; and a significant positive correlation to dough extension in the Binnu Hard and Williams Hard data sets. These relationships determined were not unexpected, and it would be predicted more extensible dough would make more extensible end products. Lastly, the work required to puncture the chapatti was determined to have a significant positive correlation to flour swelling volume and peak viscosity, and a significant negative correlation to starch pasting setback in the Binnu and Williams data sets.

Although significant relationships were identified between flour quality traits, between chapatti quality traits, and between flour and chapatti quality traits; there were few relationships across all the data sets, Indian, Binnu Hard and Williams Hard. The variation in flour and chapatti quality, influenced by genotype and environmental factors, were likely to have been responsible for this.

4.2.4 Conclusion

A range of Indian and Australian wheat varieties were stone milled to create whole wheat flours of high extraction (100% minus coarse bran particles) suitable for chapatti making. Characterisation and evaluation of physicochemical and rheological properties determined that the chapatti flour quality of the Indian and the Australian wheat varieties was not similar. The chapatti flour quality of the Australian soft noodle wheat varieties was significantly different to the Indian and the Australian hard wheat varieties. Furthermore, the Indian and the Australian hard wheat varieties also had different chapatti flour quality. The flour quality traits which were identified as being important for determining differences between the Indian and the Australian hard wheat varieties were flour colour *CIE L** and *a**, lightness and redness, particle size index and water absorption. The second set of quality traits responsible for the variation in chapatti flour quality between the wheat varieties were ash content, damaged starch content and dough extension values.

Quality chapatti flour can be characterised as having high damaged starch content and high water absorption; that interact to make dough that is not sticky during hand chapatti making. In comparison to the Indian wheat varieties, the Australian hard wheat varieties were found to have lower damaged starch content and water absorption. Flour for making quality chapatti can also be characterised as having protein content in the range of 10 to 12%, and dough that is easy to knead and sheet. Dough extensibility was determined to be significantly lower for Indian wheat varieties in contrast to the Australian hard wheat varieties so Indian wheat dough's were easier to knead and sheet by hand.

The Indian chapatti flours were also characterised as having lighter flour colour, higher *CIE L** values, with less redness and yellowness, lower *CIE a** and *b** values;

than the Australian hard wheat chapatti flours. In addition, a creamy light brown to golden colour is desirable in chapatti and contributes to quality of appearance. Australian hard wheat varieties have higher positive *CIE b** values, yellowness, in contrast to the Indian wheat varieties. Nonetheless, the ash content of the Indian chapatti flours was determined to be higher than the Australian hard wheat varieties. Therefore, flour colour measurements may be affected by other factors, than bran content and endosperm colour, and this could also include particle size index. Particle size was also identified as being an important quality difference; the Indian chapatti flours had significantly finer particle size flours than the Australian hard wheat varieties. The Australian hard wheat varieties were determined, from particle size index characterisation, to have harder grain endosperm than the Indian wheat varieties; and this helped to explain the medium hard type of grain reported for chapatti.

There were however some similarities in chapatti quality shown between the Indian and the Australian hard wheat varieties. Australian hard wheat variety *Bumper*, grown in the Binnu trial, was the most similar to the samples of Indian wheat varieties for chapatti colour *CIE L* a** and *b**, especially to *PBW 175*. The chapatti quality traits which were identified as determining differences between the Indian and the Australian hard wheat varieties were traits that related to colour and textural attributes. The first set of chapatti quality traits identified as being important included the work required to tear and puncture chapatti samples; a measure of softness, toughness and extension properties. The peak force needed to puncture a chapatti, and the lightness *CIE L** of side one of the chapatti, the side with the scattering of light brown spots and served upwards, were also important. The second combination of quality traits which explained variability between the wheat varieties highlighted lightness, *CIE L**, of side two of the chapatti; redness colour, *CIE a**, on side one and two; and the extensibility of the chapatti samples to tear and puncture, as being important. Chapatti quality of the Australian soft noodle wheat varieties however, were identified as being different to the Indian and the Australian hard wheat varieties, which further confirmed the quality requirement for hard wheat types.

Chapatti quality can be described as creamy light brown to yellow in colour with a scattering of light brown spots over the surface. Full puffing of chapatti is desired so that two distinct layers are formed. Furthermore, the texture of chapatti should be soft and pliable, being able to tear and fold easily, and not be tough or brittle. The chapatti colour of the Indian wheat varieties was collectively shown to be slightly lower in lightness, redness and yellowness, in comparison to the Australian hard wheat varieties; this trend was also seen for flour colour. Although puffed height was not shown to be important for distinguishing chapatti quality of the Indian and the Australian hard wheat varieties, it was shown to have importance differentiating wheat varieties within some data sets. Nevertheless, the chapatti samples evaluated were all shown to puff sufficiently to create two separate layers; a key characteristic of the product.

Of the Indian wheat varieties assessed, *HI 1531* and *PBW 175* were shown to have superior chapatti quality characteristics; and were used as a gold standard for comparative purposes. The chapattis of these wheat varieties were lighter in colour, and puffed higher and more fully across the entire surface area of the chapatti. Furthermore, the textural characteristics of *HI 1531* and *PBW 175* showed that the chapattis were softer and were easier to tear and puncture, in contrast to the other Indian wheat varieties. *HI 1531* and *PBW 175* had lower peak force values and required less work to tear and puncture chapatti samples. Australian hard wheat varieties identified as having similar textural properties to *HI 1531* and *PBW 175* for peak force, and work required to tear and puncture chapatti samples were; *Bumper*, *Correll*, *Espada*, *Fang*, *Gladius* and *Magenta*. In addition, the textural attribute of extensibility was shown to greater for *HI 1531* and *PBW 175* than the other Indian wheat varieties; however many Australian hard wheat varieties had similar extensibility properties, with *Bumper* closest to the Indian wheat varieties and the lowest extensibility of the Australian hard wheat varieties studied.

Significant relationships were identified between flour and chapatti quality traits in each of the data sets, however there were few relationships that were found in all of the Indian, Binnu Hard and Williams Hard data sets. The measurement of protein content, damaged starch content, water absorption, flour colour (as a predictor of chapatti colour) and dough extension (as a predictor of texture) would all assist in the screening of wheat varieties for chapatti. It appears particle size may be important so more research is needed in this area.

4.3 Evaluation of the chapatti making ability of selected Australian wheat varieties using a second chapatti making method

4.3.1 Introduction

A review of the chapatti making method used to make chapatti samples for quality evaluation, highlighted several potential issues which were not identified when the method was developed and initially tested. The issues recognised, may have had an influence on chapatti quality, and therefore potential improvements to the method were made with the assistance of expert advice. The second laboratory chapatti making method was developed with Associate Professor Hardeep Singh Gujral during his visit to Perth, W.A. in September 2010. The second method aimed to improve upon the existing chapatti making method and notable changes included; subjectively determining the optimum water absorption for chapatti making; puffing the chapatti on the electric hot plate, and not cutting the chapatti dough sheet with a template. In addition, adjustments to weights, times and temperatures were made to suit the changed conditions.

It was hypothesised that the chapatti quality of the Australian and the Indian wheat varieties would not significantly change for chapatti made from the first and the second method. Furthermore, that the relationships between flour quality and chapatti quality, and the differences between Australian and Indian wheat varieties would be similar to those previously established.

4.3.2 Materials and Methods

Summary of materials and methods

Eight Australian wheat varieties, *Bumper, Espada, Fang, Gladius, Mace, Magenta, Tammarin Rock* and *Yitpi*, were selected for evaluation using the second chapatti making method. Composite flour blends for each wheat variety were created by mixing flour from the Binnu and the Williams trials in an equal amount. The six Indian wheat varieties, *C 306*, *HI 1531*, *K 9107*, *PBW 175*, *PBW 343* and *WH 542,* were also assessed. Three chapattis were made from one dough sample and this was conducted in triplicate for each wheat variety; the samples were also tested in a randomised order. (See Table 3.5 for details of the second laboratory chapatti making method).

Statistical methods

The flour quality trait data previously collected for the selected Australian and Indian wheat varieties was used for the subsequent statistical analyses. The flour for the Australian wheat varieties was composited in an equal ratio from the Binnu and the Williams trials. Thus a mean was calculated for each flour quality trait, for each of the eight Australian wheat varieties, from the predicted means previously determined. The predicted means for the flour quality traits of the Indian wheat varieties were used as earlier described.

Predicted means and standard errors were obtained for each chapatti quality trait, for each wheat variety, by running unbalanced ANOVA on the data collected. The unbalanced ANOVA had the chapatti quality traits as the Y-variate and the treatment as ‘variety’, referring to wheat variety; there were no blocking terms used. The data sets were unbalanced due to variation in the number of wheat varieties in each data set, and variation in the number of replicate measurements performed for the different tests.

Correlations were performed to identify significant correlations between flour quality traits; between chapatti quality traits; and significant relationships between flour and chapatti quality traits. Significant correlations were identified using critical values for Pearson’s correlation coefficients at the 5% level of significance ($p<0.05$).

Principle component analysis (PCA) was performed to identify the important combinations of quality traits which were determining differences between the wheat varieties in the data sets analysed. The flour quality traits and the chapatti quality traits were analysed separately for each data set, and four principal components were generated. PCA used a correlation matrix analysis to standardise the variates as they did not all share a common scale. Biplots were produced to visually look at the first two principal components (PC-1 and PC-2) driving variation within a data set; and to compare similarities and differences between the data sets analysed.

4.3.3 Results and Discussion

Quality characterisation of selected Australian chapatti flours blended from two locations.

The chapatti flour quality of the Australian wheat varieties chosen for further study was characterised and predicted means of selected flour quality traits are shown in Table 4.25. The complete flour quality characterisation data is presented in Appendix 1.35. Blending of the chapatti flours to produce a composite sample resulted in the values of the flour quality traits to represent means of the flour quality characterisation data collected for the Australian wheat varieties from the Binnu and the Williams trials. The protein content and particle size index were particularly shown to change, as a result of blending, and ranged from 11.2 to 12.5% and 554.7 to 653.6 µm, respectively; see Table 4.25. The remaining flour quality traits characterised, for the selected Australian hard wheat varieties, were similar in value and range as they were prior to blending; see Appendix 1.24 and 1.25.

Table 4.25. Predicted means of selected flour quality traits for the Australian chapatti flours blended from two locations.

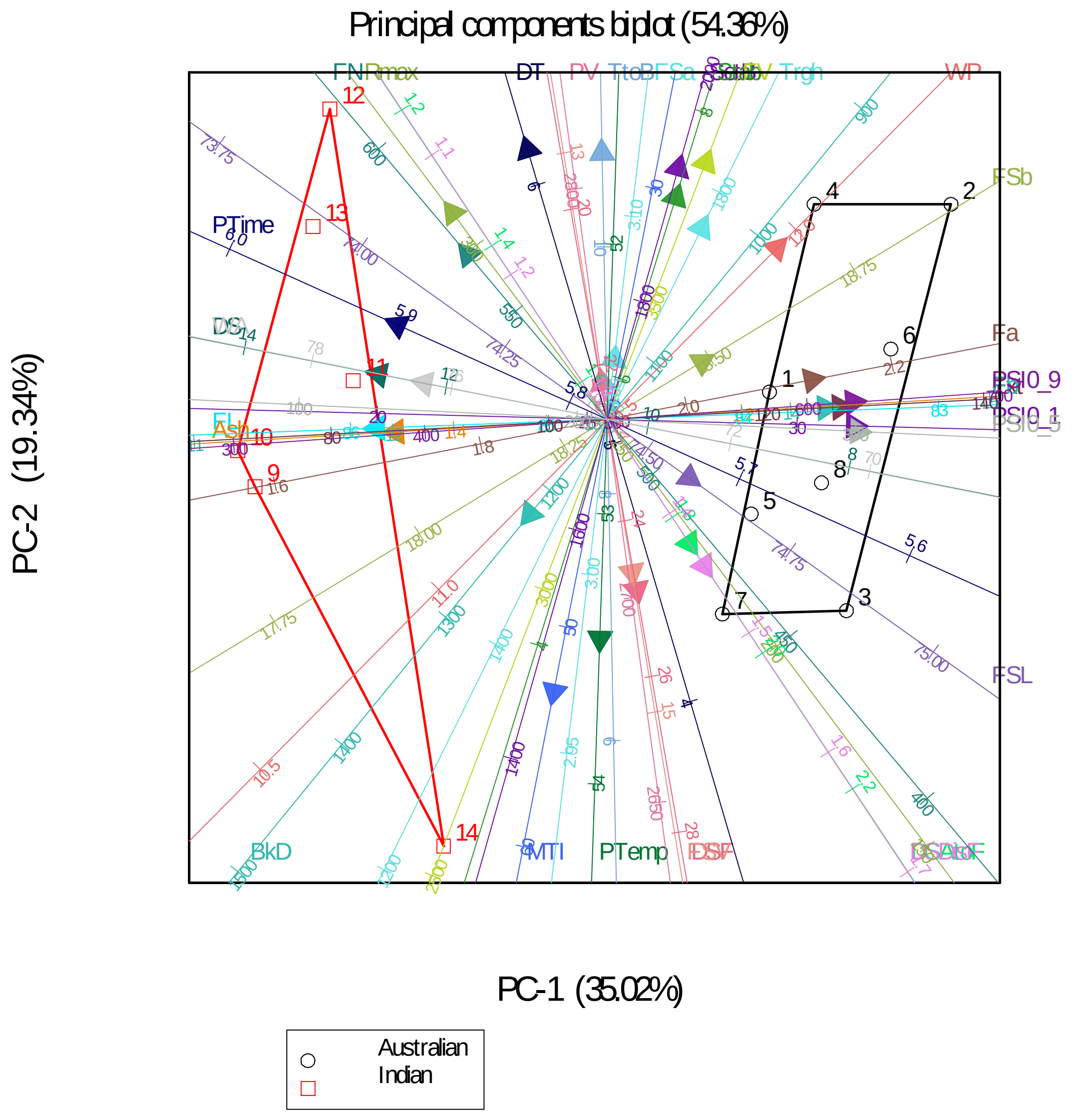
Wheat Variety	Grade	FN (sec)	Ash (%)	WP (%)	WA (%)	DS (%)	Ext (mm)	PSI0.9 (µm)
HI 1531	*Indian*	*677*	*1.30*	*11.8*	*86.0*	*14.5*	*85*	*323.0*
PBW 175	*Indian*	*610*	*1.58*	*11.0*	*82.9*	*16.0*	*78*	*333.4*
Bumper	ASW	492	1.08	11.2	72.8	8.7	110	611.9
Espada	APW	494	1.17	12.5	70.9	7.8	130	653.6
Fang	APW	486	1.25	11.8	72.0	8.6	124	633.2
Gladius	APW	495	1.08	12.0	69.4	8.5	136	586.3
Magenta	APW	490	1.19	11.8	71.6	9.1	127	626.6
Mace	AH	506	1.12	11.4	69.8	8.5	124	554.7
Tammarin Rock	AH	423	1.06	11.5	71.6	8.0	138	590.5
Yitpi	AH	485	1.14	11.8	68.8	8.0	118	643.3

LEGEND - **Ash**: ash content; **DS**: damaged starch content; **Ext**: dough extension; **FN**: falling number; **PSI0.9**: 90th percentile of the particle size distribution; **WA**: farinograph water absorption; **WP**: protein content

Comparison of the quality of the Indian chapatti flours and selected Australian chapatti flours blended from two locations.

A comparison of the chapatti flour quality of the Indian wheat varieties and the blended Australian chapatti flours was conducted. Principle component analysis (PCA) was performed to assess similarities and differences in flour quality of the chapatti flours. The PCA output with latent vector loadings is presented in Appendix 1.36. A convex hull was created around the wheat varieties, and the Indian wheat varieties are shown in red and the selected Australian hard wheat varieties in black on the biplot; see Figure 4.9.

Figure 4.9. Biplot of PCA of flour quality traits for the Indian and the Australian chapatti flours blended from two locations.



The first principal component (PC-1) accounted for 35.02% of the variability between the wheat varieties and the quality traits of particle size, dough extension, flour colour *CIE L** and *b**, lightness and yellowness, damaged starch content, and ash content were found to be important. The Indian wheat varieties are shown to be grouped on the left hand side of the biplot, Figure 4.9, and the selected Australian hard wheat varieties positioned on the right hand side of the biplot. The first principle component separated the wheat varieties across the biplot and the quality traits described above were therefore largely responsible for differentiating the flour quality of the Indian wheat varieties and the selected Australian hard wheat varieties. The Australian chapatti flours were shown to have higher particle size, dough extension and flour colour *CIE b**, yellowness, values than the Indian chapatti flours. In addition, the selected Australian hard wheat varieties also had lower values for ash content, damaged starch content and flour colour *CIE L**, lightness, in comparison to the Indian wheat varieties studied. The chapatti flour quality of the Indian and the Australian hard wheat varieties was found to be different and this also confirmed the finding from the previous study. The flour quality traits identified as important distinguishing traits in this PCA analysis, have also been previously identified as key contributing factors which differentiate the Indian and the Australian hard wheat chapatti flours.

The previous study, compared the flour quality of the Indian, the Binnu Hard and the Williams Hard wheat varieties, see Figure 4.5, and the second principle component (PC-2) was found to mainly determine differences between the Indian and the Australian hard wheat varieties. The quality traits that were important in PC-2 were ash content, damaged starch content, dough extension and starch pasting trough; quality traits which were also identified, except for starch pasting trough, in the comparison of Indian and Australian hard wheat chapatti flours in this study. The flour quality traits of particle size and flour colour *CIE L**, lightness, were identified as determining differences in PC-1 in the previous study. PC-1 was mainly responsible for distinguishing the Williams Hard wheat varieties from the Binnu Hard and the Indian wheat varieties. Blending the flour from the Williams and the Binnu trials however has resulted in particle size and flour colour *CIE L**, lightness, to be important quality differences between the Australian hard and the Indian chapatti flours studied. Thus the flour quality traits identified in PC-1 in this study, except *CIE b**, yellowness, confirmed the previous findings, as the quality traits were all earlier identified as being important in differentiating flour quality of the Indian and the Australian hard wheat varieties.

The second principle component (PC-2) explained 19.34% of the variation in flour quality of the wheat varieties; and the dough properties of mixing tolerance index, development time, and time to breakdown were shown to be important for differentiating the wheat varieties. Starch pasting properties of final viscosity and setback were also key contributing traits in PC-2. The aforementioned flour quality traits were shown to mainly differentiate the wheat varieties within each data set; the Indian and the selected Australian hard wheat varieties, based on differences in protein and starch qualities. The wheat varieties in each data set are shown in Figure 4.9 to be spaced from the top to the bottom of the biplot, and along the vectors identified to be important in PC-2.

Characterisation of chapatti quality of the Indian wheat varieties using a second chapatti making method.

The Indian wheat varieties were made into chapatti using a second chapatti making method and the chapatti quality was assessed as previously performed with addition of a sensory assessment. The results for these chapatti quality assessments are described in Appendix 1.37; and percentage bake loss, puffed height, percentage surface area of the chapatti puffed and sensory assessment score are shown in Table 4.26. The percentage bake loss ranged from 9.7 to 11.6% and these results were shown to be significantly lower than the results from the previous chapatti making method ($p<0.001$). Differences in the amount of water added to the flour and shorter chapatti cooking times may have contributed to this difference. Puffed height was generally found to be higher for the Indian wheat varieties reported to have good chapatti quality, *HI 1531*, *PBW 175* and *C 306*; see Table 4.26. A higher puffed height for wheat varieties which make good quality chapatti was expected and this observation was also seen for the first chapatti making method; except for wheat variety *C 306*. The most likely reason Indian wheat variety *C 306* did not puff well in the first chapatti making method was an insufficient water addition. The sensory assessment of chapatti was an additional quality test, and was based on an evaluation of the following attributes; percentage surface area of the chapatti puffed, colour, appearance, hand feel/ fold ability, hand tearing, aroma, taste, and mouth feel/ chewiness. The results showed that the Indian wheat varieties with good chapatti quality, *HI 1531*, *PBW 175* and *C 306*, had higher sensory assessment scores and they also puffed across a greater area of the chapatti's surface; see Table 4.26.

Table 4.26. Predicted means for the chapatti quality traits of percentage bake loss, puffed height, percentage surface area puffed, and sensory assessment score of the Indian wheat varieties using a second chapatti making method.

Wheat Variety	BL (%)	PH (mm)	SAP (%)	Sensory Assessment Score (/100)
HI 1531	10.5	48.9	86.1	80.1
PBW 175	11.2	46.1	88.9	80.5
C 306	11.6	52.2	98.6	83.1
K 9107	10.4	40.6	79.2	71.3
PBW 343	10.3	41.1	82.0	72.9
WH 542	9.7	41.7	86.1	72.3

LEGEND - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed

The colour of the raw chapatti dough sheets of the Indian wheat varieties were measured on side one and two; and predicted means for these colour measurements are displayed in Table 4.27. The colour of the raw chapatti dough sheets was shown to range from 70.57 to 73.56 for *CIE L**, lightness; from 4.38 to 5.51 for *CIE a**, redness; and from 19.63 to 23.17 for *CIE b**, yellowness. There was no significant difference between side one and side two colour measurements and this was expected. The raw chapatti dough sheet colour however, was significantly lighter ($p<0.05$) and less yellow ($p<0.05$) than the colour of the raw chapatti dough sheets made using the first chapatti making method; and this is likely due to differences in water added, mixing times and the amount of flour used for dusting.

Table 4.27. Predicted means for raw chapatti dough sheet colour measurements of the Indian wheat varieties using a second chapatti making method.

Wheat Variety	R1L (*L**)	R1a (*a**)	R1b (*b**)	R2L (*L**)	R2a (*a**)	R2b (*b**)
HI 1531	73.49	4.40	22.37	73.56	4.48	22.80
PBW 175	71.36	5.25	22.68	71.06	5.42	23.17
C 306	72.10	4.38	19.63	71.68	4.55	20.29
K 9107	72.37	4.91	20.43	71.75	5.21	21.85
PBW 343	72.87	4.54	21.05	72.59	4.61	21.62
WH 542	71.19	5.34	20.96	70.57	5.51	21.98

LEGEND - **R1L**: raw chapatti dough sheet colour *CIE L** of side one; **R1a**: raw chapatti dough sheet colour *CIE a** of side one; **R1b**: raw chapatti dough sheet colour *CIE b** of side one; **R2L**: raw chapatti dough sheet colour *CIE L** of side two; **R2a**: raw chapatti dough sheet colour *CIE a** of side two; **R2b**: raw chapatti dough sheet colour *CIE b** of side two

The colour of the baked chapattis made using the second chapatti making method were determined to generally decrease in lightness, *CIE L** value, and have both slight increases and decreases in redness and yellowness, *CIE a** and *b** values, after baking; see Table 4.28. Moreover, the colour of the baked chapattis were determined to have some similarities to the baked chapatti colour from the first chapatti making method used. However the colour measurements of *CIE L** and *a** side one ($p<0.05$), and *CIE b** side two ($p<0.05$), were identified as being significantly different to the baked chapatti colour measured from the first method. Differences in the chapatti making methods, particularly the water added and baking times and temperatures, have contributed to the differences seen in raw and baked chapatti colour of the Indian wheat varieties.

Table 4.28. Predicted means for the baked chapatti colour measurements of the Indian wheat varieties using a second chapatti making method.

Wheat Variety	B1L (*L**)	B1a (*a**)	B1b (*b**)	B2L (*L**)	B2a (*a**)	B2b (*b**)
HI 1531	70.75	4.12	22.00	72.69	3.48	22.82
PBW 175	69.71	4.68	22.21	71.53	4.30	22.12
C 306	68.76	4.44	19.21	69.95	4.23	20.97
K 9107	68.39	5.21	21.52	70.59	4.77	22.09
PBW 343	69.26	4.51	20.61	71.14	4.13	21.37
WH 542	68.01	5.11	21.02	70.30	4.92	22.22

LEGEND - **B1L**: baked chapatti colour *CIE L** of side one; **B1a**: baked chapatti colour *CIE a** of side one; **B1b**: baked chapatti colour *CIE b** of side one; **B2L**: baked chapatti colour *CIE L** of side two; **B2a**: baked chapatti colour *CIE a** of side two; **B2b**: baked chapatti colour *CIE b** of side two

The two extensibility texture tests, which involved tearing a chapatti strip and puncturing a whole chapatti, were also conducted on the chapatti samples made using the second method. Predicted means for the texture measurements taken from the two extensibility tests for the Indian wheat varieties are presented in Table 4.29. The results for the first extensibility test showed that Indian wheat variety *PBW 175* required the least amount of force and work to tear a chapatti strip, and had medium extensibility. It was observed that chapatti samples which were more extensible, sometimes also required greater force, and therefore work to tear due to their greater stretching abilities; wheat varieties *C 306*, *HI 1531* and *PBW 343* demonstrated this finding. On the other hand, *WH 542* made the toughest and least extensible chapatti with the highest force required to tear, and the lowest extensibility values; see Table 4.29. A comparison of the results for the first extensibility test, to the texture measurements obtained from chapatti made using the first method, determined that although the values in Table 4.29 were slightly higher than the previous results (Table 4.15) the results were not significantly different. Therefore the chapatti making method used did not significantly affect the textural attributes of chapatti, assessed by extensibility test one, made from the Indian wheat varieties.

For the second extensibility test, wheat variety *PBW 175* was determined to be the most extensible and required the greatest peak force and work to puncture a whole chapatti. Indian wheat variety *HI 1531* was the next most extensible and also required the second greatest amount of force and work to puncture the chapatti samples. The two aforementioned wheat varieties have both been reported to have good chapatti quality and the extension properties exhibited demonstrated this. The higher peak force and work to puncture values however appeared to indicate tougher chapatti samples, which is not desirable for chapatti, and has resulted from the chapatti making method used. In contrast to the texture results obtained for chapattis made using the first chapatti making method, the values for peak force ($p<0.05$) and work required to puncture a chapatti ($p<0.05$), were significantly higher from the second chapatti making method. The extensibility properties however were shown to be similar. The second chapatti making method, with different baking times and temperatures, was thought to have created a harder crust across the entire surface of the chapatti thus making it harder to penetrate with the spherical probe.

Table 4.29. Predicted means for texture measurements taken from two extensibility tests for the Indian wheat varieties using a second chapatti making method.

Wheat Variety	E1PF (g)	E1Dis (mm)	E1A12 (g.s)	E2PF (g)	E2Dis (mm)	E2A12 (g.s)
HI 1531	678.70	6.27	2900	1097	21.45	8754
PBW 175	538.10	5.67	2039	1290	22.35	10276
C 306	687.30	7.95	3821	1072	18.82	6799
K 9107	661.00	4.50	2156	966	19.14	6900
PBW 343	767.10	5.87	2992	1073	20.31	6985
WH 542	854.80	3.51	2082	1129	20.40	8214

LEGEND - **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti

Comparison of the quality of chapattis made using chapatti making method one and two for the Indian wheat varieties.

Chapattis were made using the same Indian chapatti flours for the two different chapatti making methods. Therefore a comparison of chapatti quality of the Indian wheat varieties was conducted. The key differences between the two chapatti making methods were related to changes in the amount of water added to the flour, and the baking times and temperatures. The water added in the second method was observed to be closer to the optimum water addition needed, but although the procedure was standardised, it was required to be subjectively determined. Furthermore, the baking temperature used in the second method was almost 100 ºC higher and consequently the baking times were shorter, than the temperature and times used in the first method. The chapattis were also puffed on the hotplate, as oppose to the oven which was used in the first method. Differences in chapatti quality have resulted from the different methods used, and some of the effects on chapatti quality have been already described. Changes in the appearance of the chapattis were apparent and can be seen in Figures 4.10 and 4.11. For example, the brown spots on the chapattis made using the second method were darker and smaller in comparison to the chapattis made using the first method; and overall the chapattis were not as golden brown for Indian wheat variety *PBW 175* when made using the second method.

Figure 4.10. Chapatti samples of Indian wheat variety *PBW 175* made using the first laboratory chapatti making method.

Side 1

Side 2

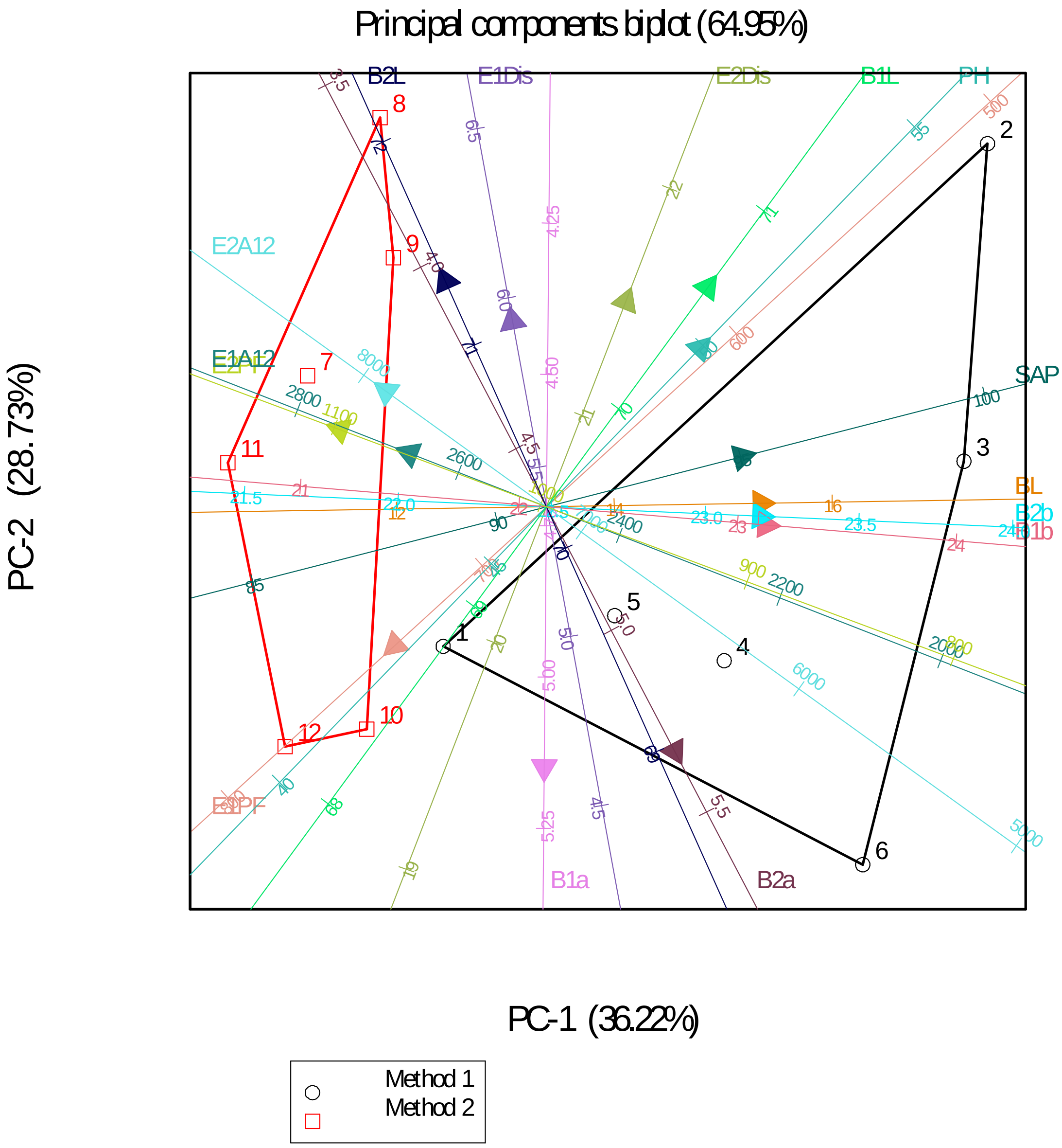
Figure 4.11. Chapatti samples of Indian wheat variety *PBW 175* made using the second laboratory chapatti making method.

Side 1 **Side 2**

PCA was performed to compare the chapatti quality of the Indian wheat varieties made using the two different methods and help to further understand the effect of the chapatti making methodology on chapatti quality. A biplot of this analysis is shown in Figure 4.12; and the PCA output is provided in Appendix 1.38. The Indian wheat varieties made into chapatti using the first chapatti making method are shown in black, and the Indian wheat varieties made into chapatti using the second chapatti making method in red on the biplot.

The first principal component (PC-1) explained 36.22% of the variability between the Indian wheat varieties based on chapatti quality. PC-1 was shown to separate the Indian wheat varieties based on the chapatti making method used. The Indian wheat varieties made into chapatti using the first method can be seen on the right hand side of the biplot (black); and the Indian wheat varieties made into chapatti using the second chapatti making method are on the left hand side of the biplot (red); see Figure 4.12. The chapatti making method used therefore effects chapatti quality. The chapatti quality traits of chapatti colour *CIE b**, yellowness, for side one and two, percentage bake loss, the peak force to puncture a chapatti, and percentage surface area of the chapatti puffed, were identified as being important in determining these quality differences. The Indian wheat varieties made using the second chapatti making method had reduced yellowness, *CIE b**, side one and two, lower percentage bake loss, decreased percentage surface area of the chapatti puffed, and increased peak force to puncture a chapatti; in comparison to the chapattis made using the first method.

Figure 4.12. Biplot of PCA of chapatti quality traits for the Indian wheat varieties using two different chapatti making methods.



Nonetheless, despite these differences the application of a standard chapatti making method was shown to differentiate wheat varieties based on chapatti quality, which was a desired outcome. The second principle component (PC-2) was shown to separate the Indian wheat varieties based on chapatti quality, and accounted for 28.73% of the variation in chapatti quality between the wheat varieties. The Indian wheat varieties are seen to be separated from the top to the bottom of the biplot, Figure 4.12, and the vectors mainly responsible for this were chapatti colour *CIE L** and *a**, lightness and redness, for side one and two; and the distance a punctured chapatti was extended.

In addition, the Indian wheat varieties were determined to have similar chapatti quality, when made using either chapatti making method, and this is shown by their similar position on the biplot; see Figure 4.12. For example, Indian wheat variety *HI 1531* (2 black and 8 red) is at the top of the biplot; the next lowest wheat variety slightly to the right is *PBW 175* (3 black and 9 red); and at the bottom of the biplot is *WH 542* (6 black and 12 red). The chapatti quality appears to be of better quality towards the top of the biplot and poorer chapatti quality towards the bottom of the biplot.

Characterisation of chapatti quality of selected Australian chapatti flours blended from two locations using a second chapatti making method.

The chapatti quality of the selected Australian hard wheat varieties was characterised and is described in Appendix 1.39. Predicted means for percentage bake loss, puffed height, percentage surface area of the chapatti puffed, and sensory assessment scores are shown in Table 4.30. The percentage bake loss ranged from 8.33 to 9.31% and was generally lower in value than the percentage bake loss from the first chapatti making method for the Australian hard wheat varieties. The shorter baking time used in the second chapatti making method and different amount of water added to the flour were likely to have contributed to this. Furthermore, the percentage bake loss of the selected Australian hard wheat varieties was significantly lower than the Indian wheat varieties ($p<0.001$) using the second chapatti making method.

Puffed height of the chapattis was determined to range from 17.8 to 46.1 mm, with three of the eight Australian hard wheat varieties having a puffed height of less than 30 mm. A low puffed height is not a desirable quality characteristic of chapatti. Moreover, *Espada*, *Gladius* and *Yitpi*, which had the lower puffed height values, also had lower results for the percentage surface area of the chapatti puffed; see Table 4.30. The percentage surface area of the chapatti puffed was less than 60% for these three Australian hard wheat varieties, indicating poor chapatti quality for this trait. A comparison to results, for Australian hard wheat varieties, from the first chapatti making method, identified that the values for puffed height and percentage surface area of the chapatti puffed, were not previously as low. A possible explanation for these differences in puffing abilities may be due to the different puffing technique used in the second method. The second chapatti making method puffed the chapattis at the same temperature they were baked at on the hotplate; whereas in the first method the chapattis were puffed in an oven with a significant increase in temperature. The change in temperature would have created an instantaneous and greater generation of steam in the first method. The Australian hard wheat varieties may have been needed this action to overcome their stronger protein and therefore it allowed the chapattis to puff more fully and higher. Nevertheless, although in the second chapatti making method puffed height and percentage surface area of the chapattis puffed were lower than the first method, the results were not significantly lower than the Indian wheat varieties. Therefore the Indian wheat varieties also did not puff as well as they did in the first chapatti making method. The only wheat varieties which performed well in both methods were Indian wheat varieties *HI 1531* and *PBW 175*, and Australian hard wheat variety *Bumper* and this may indicate these wheat varieties have weaker protein.

The sensory assessment scores were determined to range from 47.9 to 73.5 for the Australian hard wheat varieties, and are displayed in Table 4.30. The sensory assessment was a subjective evaluation of the chapatti quality of the wheat varieties studied. The results showed that four wheat varieties *Mace*, *Magenta*, *Bumper* and *Tammarin Rock* had a sensory assessment score which ranged from 70 to 75; where the higher the score the better chapatti quality. These Australian hard wheat varieties therefore had acceptable chapatti quality. The sensory assessment scores for these Australian wheat varieties were similar to the Indian wheat varieties *K 9107*, *PBW 343* and *WH 542*; see Table 4.30. The remaining four Australian hard wheat varieties however had scores lower than 70. Consequently, the sensory assessment scores of the Australian hard wheat varieties were determined to be significantly lower than the Indian wheat varieties ($p<0.05$), and generally indicated lower chapatti quality.

Table 4.30. Predicted means for the chapatti quality traits of percentage bake loss, puffed height, percentage surface area puffed, and sensory assessment score of the Australian chapatti flours blended from two locations using a second chapatti making method.

Wheat Variety	Grade	BL (%)	PH (mm)	SAP (%)	Sensory Assessment Score (/100)
HI 1531	*Indian*	*10.5*	*48.9*	*86.1*	*80.1*
PBW 175	*Indian*	*11.2*	*46.1*	*88.9*	*80.5*
K 9107	*Indian*	*10.4*	*40.6*	*79.2*	*71.3*
PBW 343	*Indian*	*10.3*	*41.1*	*82.0*	*72.9*
WH 542	*Indian*	*9.7*	*41.7*	*86.1*	*72.3*
Bumper	ASW	9.23	45.6	86.1	72.1
Espada	APW	8.33	27.8	52.8	57.6
Fang	APW	9.12	43.3	70.8	66.5
Gladius	APW	8.70	27.8	59.7	62.0
Magenta	APW	9.13	46.1	83.3	72.8
Mace	AH	9.31	42.8	84.7	73.5
Tammarin Rock	AH	8.75	45.0	84.7	71.9
Yitpi	AH	8.75	17.8	26.4	47.9

LEGEND - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed

Colour measurements were taken for the raw chapatti dough sheets of the Australian hard wheat varieties, and predicted means for these colour measurements are presented in Table 4.31. The *CIE L** values, indicating lightness, ranged from 71.13 to 73.47 for both side one and two. The *CIE a** and *b** values, representing redness and yellowness, ranged from 4.88 to 5.68 and 20.75 to 24.82; respectively. The raw chapatti dough sheet colour results were contrasted to those previously obtained for Australian hard wheat varieties made into chapattis using the first chapatti making method; and it was observed that the lightness was similar, but the redness and yellowness were slightly lower using the second method. Nonetheless, in comparison to the Indian wheat varieties, the raw chapatti dough sheet colour of the selected Australian hard wheat varieties had greater redness and yellowness, and this was significantly higher for side one ($p<0.05$).

Table 4.31. Predicted means for raw chapatti dough sheet colour measurements of Australian chapatti flours blended from two locations using a second chapatti making method.

Wheat Variety	Grade	R1L (L^*)	R1a (a^*)	R1b (b^*)	R2L (L^*)	R2a (a^*)	R2b (b^*)
HI 1531	*Indian*	*73.49*	*4.40*	*22.37*	*73.56*	*4.48*	*22.80*
PBW 175	*Indian*	*71.36*	*5.25*	*22.68*	*71.06*	*5.42*	*23.17*
Bumper	ASW	72.21	5.52	21.17	71.90	5.65	21.18
Espada	APW	71.13	5.55	24.32	71.35	5.44	24.68
Fang	APW	72.83	5.33	22.81	73.04	5.41	23.46
Gladius	APW	73.19	5.00	23.38	73.06	4.88	23.79
Magenta	APW	71.39	5.54	24.75	71.23	5.68	24.82
Mace	AH	72.54	5.19	23.42	72.03	5.39	24.34
Tammarin Rock	AH	73.39	5.02	20.75	73.47	5.04	20.81
Yitpi	AH	73.30	4.92	22.77	72.30	5.11	23.83

LEGEND - **R1L**: raw chapatti dough sheet colour *CIE* L^* of side one; **R1a**: raw chapatti dough sheet colour *CIE* a^* of side one; **R1b**: raw chapatti dough sheet colour *CIE* b^* of side one; **R2L**: raw chapatti dough sheet colour *CIE* L^* of side two; **R2a**: raw chapatti dough sheet colour *CIE* a^* of side two; **R2b**: raw chapatti dough sheet colour *CIE* b^* of side two

Colour of the baked chapattis were also measured and predicted means for the colour measurements of the Australian hard wheat varieties are shown in Table 4.32. It was generally observed that baking of chapattis resulted in a decrease in the lightness, redness and yellowness, in comparison to the raw chapatti dough colour. A similar trend was also seen when chapattis were made using the first chapatti making method, though the percentage change in *CIE* L^* a^* and b^* values, was noted to vary for different wheat varieties. The colour of the baked chapattis of the selected Australian hard wheat varieties, although slightly redder and yellower; were not found to be significantly different to the Indian wheat varieties.

Table 4.32. Predicted means for the baked chapatti colour measurements of Australian chapatti flours blended from two locations using a second chapatti making method.

Wheat Variety	Grade	B1L (L^*)	B1a (a^*)	B1b (b^*)	B2L (L^*)	B2a (a^*)	B2b (b^*)
HI 1531	*Indian*	*70.75*	*4.12*	*22.00*	*72.69*	*3.48*	*22.82*
PBW 175	*Indian*	*69.71*	*4.68*	*22.21*	*71.53*	*4.30*	*22.12*
Bumper	ASW	68.62	5.06	19.73	70.11	4.73	20.82
Espada	APW	68.02	5.14	23.02	69.82	4.74	22.92
Fang	APW	70.59	4.66	21.65	71.45	4.58	22.94
Gladius	APW	68.33	5.02	22.90	70.41	4.41	22.49
Magenta	APW	69.06	4.94	23.13	70.73	4.60	23.94
Mace	AH	67.76	5.14	22.19	69.57	4.69	23.24
Tammarin Rock	AH	69.42	4.82	20.37	71.34	4.41	20.97
Yitpi	AH	69.67	4.76	21.83	69.11	4.90	22.97

LEGEND - **B1L**: baked chapatti colour *CIE* L^* of side one; **B1a**: baked chapatti colour *CIE* a^* of side one; **B1b**: baked chapatti colour *CIE* b^* of side one; **B2L**: baked chapatti colour *CIE* L^* of side two; **B2a**: baked chapatti colour *CIE* a^* of side two; **B2b**: baked chapatti colour *CIE* b^* of side two

Textural attributes of the selected Australian hard wheat varieties were measured using two different extensibility tests and predicted means of the texture measurements obtained are presented in Table 4.33. The first extensibility test involved stretching a chapatti strip until it was torn. The peak force to tear a chapatti strip ranged from 535.10 to 676.40 g; the extensibility ranged from 3.57 to 7.43 mm; and the work to tear a chapatti strip ranged from 1657 to 3398 gs^{-1}. *Yitpi* was shown to be the most extensible, while *Magenta* was the least extensible; and *Bumper* required the least amount of force to tear a chapatti strip and therefore had softer texture; see Table 4.33. The results attained were not dissimilar to earlier reported texture measurements for Australian hard wheat varieties made into chapatti using the first method; and which were also shown to vary for different wheat varieties. The textural attributes of the selected Australian hard wheat varieties were not found to be significantly different to the Indian wheat varieties for extensibility test one.

Table 4.33. Predicted means for texture measurements taken from two extensibility tests for Australian chapatti flours blended from two locations using a second chapatti making method.

Wheat Variety	Grade	E1PF (g)	E1Dis (mm)	E1A12 (g.s)	E2PF (g)	E2Dis (mm)	E2A12 (g.s)
HI 1531	*Indian*	*678.70*	*6.27*	*2900*	*1097*	*21.45*	*8754*
PBW 175	*Indian*	*538.10*	*5.67*	*2039*	*1290*	*22.35*	*10276*
Bumper	ASW	535.10	5.34	2047	818	20.78	5970
Espada	APW	650.90	6.52	2981	937	23.04	6846
Fang	APW	611.20	4.65	1923	1016	24.14	7699
Gladius	APW	624.50	5.97	2413	1102	23.59	8959
Magenta	APW	676.40	3.57	1657	1086	21.36	7988
Mace	AH	650.10	7.20	3398	985	21.64	7347
Tammarin Rock	AH	591.00	4.72	1985	965	21.32	7206
Yitpi	AH	578.10	7.43	3047	951	23.27	7489

LEGEND - **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti

The second extensibility test, involved puncturing a whole chapatti with a spherical probe and the peak force required to do this was determined to range from 818 to 1102 g. The distance the centre of the chapatti stretched ranged from 20.78 to 24.14 mm; and the work required to puncture the chapatti ranged from 5970 to 8959 gs^{-1}. Australian hard wheat variety *Fang* was identified as being the most extensible, and *Bumper* was the least extensible. In addition, *Bumper* required the least amount of force to puncture the chapatti, making it the softest in chapatti texture of the wheat varieties evaluated for both extensibility tests. The selected Australian hard wheat varieties were shown to require significantly less force to puncture the chapatti samples ($p<0.05$), and were also able to extend significantly further ($p<0.05$) than the Indian wheat varieties for extensibility test two. Therefore it may be concluded that overall the chapattis of the selected Australian hard wheat varieties were softer and more extensible than the Indian wheat varieties when made using the second chapatti making method and assessed using extensibility test two.

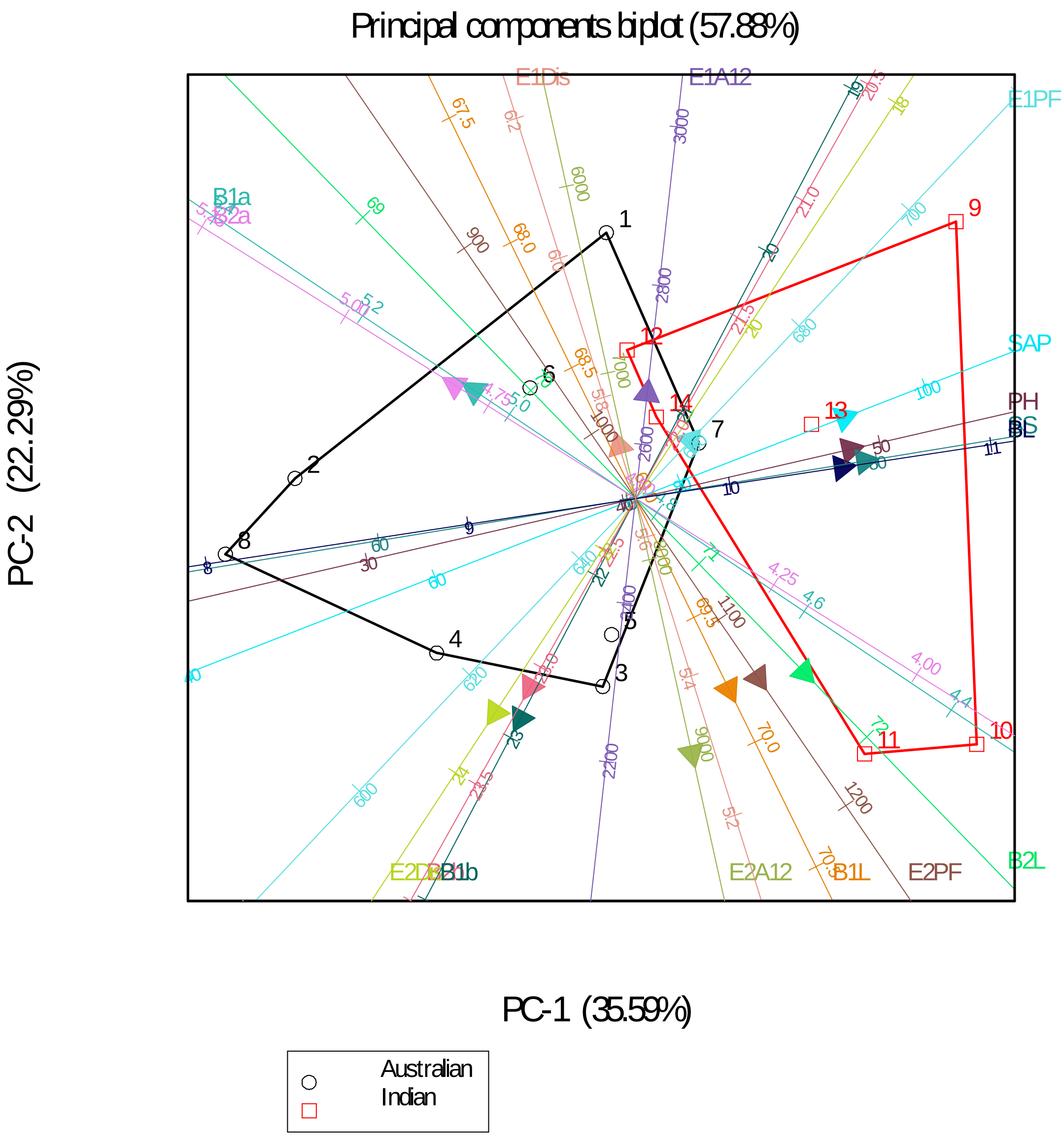
Comparison of the chapatti quality of the Indian and selected Australian chapatti flours blended from two locations using a second chapatti making method.

The chapatti quality of the Indian and selected Australian hard wheat varieties, made into chapatti using the second chapatti making method, was compared. PCA was performed to gain an understanding of the chapatti quality traits responsible for determining differences between wheat varieties. The biplot from this analysis is displayed as Figure 4.13 and the PCA output is provided in Appendix 1.40. The Australian wheat varieties are shown in black, with a convex hull drawn around them on Figure 4.13, and the Indian wheat varieties are shown in red on the biplot.

The first principle component (PC-1) explained 35.59% of the variability between wheat varieties. The chapatti quality traits highlighted as being important were the sensory assessment score, puffed height, percentage surface area of the chapatti puffed, percentage bake loss and chapatti colour *CIE a**, redness, for side two. Figure 4.13 shows PC-1 determining differences between wheat varieties across the biplot, primarily driven by the aforementioned quality traits, which are depicted as vectors. The first principal component has largely separated the Indian wheat varieties, which are positioned on the right hand side of the biplot, and the Australian hard wheat varieties, shown on the left hand side of the biplot. The Indian wheat varieties were shown to have higher sensory assessment scores, higher puffed height and percentage surface area of the chapatti puffed values, higher percentage bake loss and lower chapatti colour *CIE a**, redness; in comparison to the selected Australian hard wheat varieties. The findings suggest wheat varieties positioned further to the right hand side of the biplot have better chapatti quality than those situated towards the left hand side of the biplot. Therefore the Indian wheat varieties generally had better chapatti quality than the Australian hard wheat varieties evaluated.

The second principle component (PC-2) accounted for 22.29% of the variation between wheat varieties. Chapatti textural measurements from extensibility test two, the peak force and work required to puncture a chapatti, and the distance the chapatti extended to before puncturing, were identified as being important for differentiating wheat varieties. Chapatti colour *CIE b**, yellowness, for side one and two, and *CIE L**, lightness, for side one were also key quality traits. PC-2 mainly separated the wheat varieties within the data sets from the top to the bottom of the biplot; see Figure 4.13. Wheat varieties towards the top of the biplot were identified as having better chapatti quality than the wheat varieties positioned around the centre or bottom of the biplot; largely due to the lower values for textural traits such as peak force and work required to puncture a chapatti, thus indicating soft textured chapatti which is desired.

Figure 4.13. Biplot of PCA of chapatti quality traits for the Indian and the Australian chapatti flours blended from two locations using a second chapatti making method.



The chapatti quality of the Indian and the Australian hard varieties was largely shown to be different and this was evident from the minimal overlap of the Indian and the Australian hard wheat varieties on the biplot, Figure 4.13. There was however some clustering of Indian and Australian hard wheat varieties, which can be observed just above the centre of the biplot. The three wheat varieties in this location were shown to have similar values for chapatti quality traits determined to be important in PC-1 and PC-2. Two Indian wheat varieties *WH 542* (14) and *K 9107* (12), and one Australian hard wheat variety *Tammarin Rock* (7) were shown to have similar chapatti quality and were considered to have average chapatti quality based on the chapatti quality traits assessed. The Australian wheat varieties positioned on the left hand side, bottom area of the biplot were considered to have poorer chapatti quality; as they had low values for sensory assessment scores, puffed height, percentage surface area of the chapatti puffed, and higher values for textural traits which indicated tougher chapatti and which is not desirable.

The Indian wheat variety *C 306* (9), top right hand side, and the Australian hard wheat variety *Bumper* (1) centre top of biplot, were identified as having better chapatti quality than the other Indian and Australian hard wheat varieties evaluated; respectively. In addition, *C 306* had better chapatti quality than *Bumper*, as the sensory assessment score, puffed height and percentage surface area of the chapatti puffed, results were higher indicating good quality chapatti. Nonetheless, these two wheat varieties were shown to have similar baked chapatti colour *CIE* L^* a^* and b^* values, for lightness, redness and yellowness.

Furthermore, although Indian wheat varieties *HI 1531* (10) and *PBW 175* (11), on the bottom right hand side of the biplot, were previously identified as having better chapatti quality than the other wheat varieties evaluated. The chapatti quality results in this study were conflicting; *HI 1531* and *PBW 175* were shown to have high values for sensory assessment score, puffed height and percentage surface area of the chapatti puffed, which indicated good quality chapatti; but also had high values for the peak force and work required to puncture a whole chapatti indicating a tougher texture which is undesirable. These two Indian wheat varieties were also determined to have higher *CIE* L^* and b^* values, lightness and yellowness, than other Indian wheat varieties and were more extensible. There were no Australian hard wheat varieties studied which had similar chapatti quality to *HI 1531* and *PBW 175*. However *Fang* (3) and *Magenta* (5) were shown to have some similarities to these Indian wheat varieties for selected chapatti quality traits; and this is demonstrated by their position on the biplot (black centre bottom).

The use of two different chapatti making methods has highlighted the differences in flour quality between the Indian and the Australian hard wheat varieties, and the effect of the chapatti making methodology on chapatti quality traits. The Australian hard wheat varieties performed differently to the Indian wheat varieties using the second chapatti making method; and it appears that the Australian hard wheat varieties need a chapatti making method more similar to the first chapatti making method to make better quality chapatti.

Relationships between flour, chapatti, and flour and chapatti quality traits for the Indian and selected Australian hard wheat varieties using a second chapatti making method.

Correlations were performed to assess relationships between flour quality traits for the selected Australian hard wheat varieties. The flour quality of the Indian wheat varieties however had not changed, and as these relationships have been previously investigated they were not repeated. Correlations were also conducted between the chapatti quality traits and the flour and chapatti quality traits in both data sets, Indian and selected Australian hard wheat varieties. Each data set was analysed separately and Appendix 1.41 contains the correlation data for the Indian and the Australian hard wheat varieties; significant positive correlations (red) and significant negative correlations (green) have been highlighted.

Correlations between flour quality traits

Significant relationships between flour quality traits ($p<0.05$) for the Australian hard wheat varieties were identified, see Table 4.34, and largely confirmed previous findings. Flour quality traits which were functions of each other, for example, starch pasting final viscosity, setback and trough were found to be significantly and positively correlated for the Australian hard wheat varieties. Also the flour quality traits which assessed similar flour quality attributes, such as dough development time, dough stability and time to breakdown from the farinograph, were shown to have a significant positive correlation with resistance to extension from the extensigraph; refer to Table 4.34. There were no significant correlations identified that were unexpected between the flour quality traits; however protein content was noted to be correlated with more flour quality traits than previously determined. Protein content was one of the main variables which changed due to flour blending and was found to have significant positive correlations with flour and flour slurry colour *CIE b**, yellowness; and significant negative correlations with starch pasting peak viscosity, breakdown, pasting temperature, and flour swelling volume. The significant correlations identified between protein content and these flour quality attributes above were not previously seen.

Table 4.34. Significant relationships (p<0.05) between flour quality traits for the selected Australian hard wheat varieties.

Quality Trait[1]	AUSTRALIAN HARD Positive correlation	AUSTRALIAN HARD Negative correlation
Ash	FSb, Fb, PSI0.5	
BkD	FSV, PTemp, PV,	FSb, Fb, SetB, WP
DS	DSAtoF, DSF	
DSAtoF	DSDisF, DSF, FN, PTime, Trgh	
DSDisF	DSF, FN, PTime, Trgh	
DSF	FV, PTime, Trgh	
DT	Rmax, Stab, TtoB	MTI
Ext		PTemp, PV, Trgh
FL	FSL, FSV	Fa, Fb, PSI0.1, PSI0.5, PSI0.9
FN	FV, SetB, Trgh	
FSL		FSa, Fa, PSI0.1
FSV	PV	FSb, Fb, PSI0.1, SetB, WP
FSa	Fa, WA	
FSb	FV, Fb, SetB, WP	
FV	Fb, SetB, Trgh	
Fa	PSI0.1, PSI0.5, WA	PTime
Fb	PSI0.5, SetB, WP	
MTI		Rmax, Stab, TtoB

PSI0.1	PSI0.5, PSI0.9	PTime
PSI0.5	PSI0.9	PTime
PSI0.9		PTime
PTemp	PV	TtoB, WP
PTime	Trgh	
PV		WP
Rmax	Stab, TtoB	
Stab	TtoB	

[1]**LEGEND** - **Ash**: ash content; **Bkd**: starch pasting breakdown; **DS**: damaged starch content; **DSAtoF**: dough stickiness area under the curve to peak force; **DSDisF**: dough stickiness distance to peak force; **DSF**: dough stickiness peak force; **DT**: dough development time; **Ext**: dough extension; **FL**: flour colour *CIE L**; **FN**: falling number; **FSL**: flour slurry colour *CIE L**; **FSV**: flour swelling volume; **FSa**: flour slurry colour *CIE a**; **FSb**: flour slurry colour *CIE b**; **FV**: starch pasting final viscosity; **Fa**: flour colour *CIE a**; **Fb**: flour colour *CIE b**; **MTI**: farinograph mixing tolerance index; **PSI0.1**: 10^{th} percentile of the particle size distribution; **PSI0.5**: 50^{th} percentile of the particle size distribution; **PSI0.9**: 90^{th} percentile of the particle size distribution; **PTemp**: starch pasting temperature; **PTime**: starch pasting peak time; **PV**: starch pasting peak viscosity; **Rmax**: dough maximum resistance to extension; **SetB**: starch pasting setback; **Stab**: farinograph dough stability; **Trgh**: starch pasting trough; **TtoB**: farinograph dough time to breakdown; **WA**: farinograph water absorption; **WP**: protein content.

Correlations between chapatti quality traits

The different chapatti making method used was determined to have affected the chapatti quality of the wheat varieties studied. Significant correlations between chapatti quality traits in both data sets, Indian and selected Australian hard wheat varieties, have been described in Table 4.35. The common significant correlations for both the Indian and the Australian hard wheat varieties are discussed. Baked chapatti colour *CIE a**, redness, was determined to have a significant negative correlation with baked chapatti colour *CIE L**, lightness. In addition, baked chapatti colour *CIE b**, yellowness, was significantly and positively correlated with raw chapatti dough sheet colour *CIE b**, and the work required to puncture a chapatti. There were minimal significant correlations between raw chapatti dough sheet colour and baked chapatti colour, which were previously observed. The correlations between the colour traits may be a consequence of inherent genetic differences between wheat varieties.

In regards to the chapatti quality trait of percentage bake loss, this was shown to have significant positive correlations with puffed height and sensory assessment score. Moreover, puffed height had significant positive correlations with sensory assessment score and percentage surface area of the chapatti puffed. Thus chapatti which puffed higher, lost a greater amount of water as steam which aided puffing; and also had a greater percentage of the surface area of the chapatti puffed and a higher sensory assessment score, which indicated better quality chapatti.

Table 4.35. Significant correlations (p<0.05) between chapatti quality traits for the Indian and selected Australian hard wheat varieties.

Quality Trait[1]	INDIAN Positive correlation	AUSTRALIAN HARD Positive correlation	INDIAN Negative correlation	AUSTRALIAN HARD Negative correlation
B1L	B2L, P2a, R1b, R2L		B1a, B2a	B1a
B1a	B2a, R1a, R2a		E1Dis, P1b, R2L, SS	
B1b	B2L, B2b, E2A12, E2Dis, P2a, R1b, R2b	B2b, E1PF, E2A12, E2PF, P2b, R1b, R2b	E1A12, P2L	
B2L	E2A12, E2Dis, P2a, R1b, R2b		B2a	B2a, E1A12, E1Dis
B2a	R1a, R2a		R1L, R2L	
B2b	P2a, R1b, R2b	E1PF, R1b, R2b		
BL	E1Dis, PH, SAP, SS	PH, SS		
E1A12	E1Dis, P2L	E1Dis, P2L	R1a, R2a, R2b	
E1Dis	PH, SS	P2L	R1a, R2a	P1a, P2a, PH
E1PF		E2PF, R1b, R2b		
E2A12	E2Dis, E2PF, P1a, P2a, P2b, R1b, R2b	E2PF	P1L, P2L	
E2Dis	E2PF, P1a, P2a, P2b, R1b, R2b		P1L, P2L	SAP, SS
E2PF	P1a, P2b		P1L, P2L	
P1L	P2L	P2L	P1a, P2a, P2b, R1b	P1a
P1a	P2a, R1b, R2b	P1b, P2a, R1a, R2a	P2L	P2L
P1b		R1a, R2a		R1L, R2L
P2L		R1L	P2b, R1a, R1b, R2a, R2b	P2a, R1a, R2a
P2a	R1b, R2b	PH, R1a, R2a, SAP, SS		

P2b	R1b, R2b	R1b, R2b		
PH	SAP, SS	SAP, SS		
R1L	R2L	R2L	R1a, R2a	R1a, R2a
R1a	R2a	R2a	R2L	R2L
R1b	R2b	R2b		
R2L			R2a	R2a
SAP	SS	SS		

[1]**LEGEND** - **BL**: percentage bake loss; **PH**: puffed height; **SAP**: percentage surface area of the chapatti puffed; **B1L**: baked chapatti colour *CIE L** of side one; **B1a**: baked chapatti colour *CIE a** of side one; **B1b**: baked chapatti colour *CIE b** of side one; **B2L**: baked chapatti colour *CIE L** of side two; **B2a**: baked chapatti colour *CIE a** of side two; **B2b**: baked chapatti colour *CIE b** of side two; **E1PF**: extensibility test one peak force to tear chapatti strip; **E1Dis**: extensibility test one distance to tear chapatti strip; **E1A12**: extensibility test one work required to tear chapatti strip; **E2PF**: extensibility test two peak force to puncture chapatti; **E2Dis**: extensibility test two distance to puncture chapatti; **E2A12**: extensibility test two work required to puncture chapatti; **R1L**: raw chapatti dough sheet colour *CIE L** of side one; **R1a**: raw chapatti dough sheet colour *CIE a** of side one; **R1b**: raw chapatti dough sheet colour *CIE b** of side one; **R2L**: raw chapatti dough sheet colour *CIE L** of side two; **R2a**: raw chapatti dough sheet colour *CIE a** of side two; **R2b**: raw chapatti dough sheet colour *CIE b** of side two; **P1L**: percentage change in chapatti colour from raw to baked of *CIE L** of side one; **P1a**: percentage change in chapatti colour from raw to baked of *CIE a** of side one; **P1b**: percentage change in chapatti colour from raw to baked of *CIE b** of side one; **P2L**: percentage change in chapatti colour from raw to baked of *CIE L** of side two; **P2a**: percentage change in chapatti colour from raw to baked of *CIE a** of side two; **P2b**: percentage change in chapatti colour from raw to baked of *CIE b** of side two; **SS**: sensory assessment score

Correlations between flour and chapatti quality traits

Lastly, correlations between flour and chapatti quality traits were determined and were shown to vary between the Indian wheat varieties and the Australian hard wheat varieties; see Table 4.36. There were some significant relationships between flour and chapatti quality traits that were present in both of the data sets, and included the significant negative correlation between the distance and work required to tear a chapatti strip and flour colour *CIE a**, redness. Furthermore, there was a significant negative correlation between baked chapatti colour *CIE L**, lightness, and dough development time and time to breakdown. A significant positive correlation was identified between baked chapatti colour *CIE a** and dough stability; and there was also a significant positive correlation between baked chapatti colour *CIE b**, yellowness, and flour slurry colour *CIE b** and protein content. Moreover, raw chapatti dough colour *CIE L* a** and *b** had significant positive correlations to flour slurry *CIE L* a** and *b**; respectively. The relationships between colour traits and dough properties and chapatti textural traits may have been influenced by different ratios of bran and endosperm in the samples impacting on flour and chapatti quality.

In addition, as colour is predominantly a genetically determined trait it may be an indicator for other genotype differences between the wheat varieties studied, such as grain hardness which could be influencing differences in flour and chapatti quality (Pasha, Anjum, and Morris 2010; Zhang et al. 2009).

Table 4.36. Significant correlations ($p<0.05$) between flour and chapatti quality traits for the Indian and selected Australian hard wheat varieties.

Quality Trait[1]	INDIAN Positive correlation	AUSTRALIAN HARD Positive correlation	INDIAN Negative correlation	AUSTRALIAN HARD Negative correlation
Ash		B2b, P1a		P1L
B1L	FSb, WA	MTI	DT, Fa, PTime, TtoB	DT, Rmax, Stab, TtoB
B1a	FSa, Fa, PTime, Stab	DT, Stab, TtoB		MTI
B1b	Ext, FL, FN, FSb, WP	FSb, Fb, SetB, WP	PTemp	BkD, FSV
B2L	Ext, FL, FN, FSb		BkD, DT, Rmax, TtoB	
B2a	DT, FSa, Fa, PTime, Stab		FSL	Ext
B2b	FL, FN, Trgh, WP	FSb, FV, Fb, SetB	PTemp	
BL		BkD, DS, DSF, PTemp, PV		WP
BkD			R1L, R2L	P2b, R1b
DS	PH, SS	P2a	E1PF, P1L	
DSAtoF	E1A12, E1Dis, P2L		R1a, R2a	
DSDisF	E1A12, E1Dis, P2L		R1a, R2a	
DSF	E1Dis		R1a, R2a	
E1A12	PSI0.5		Fa	Fa, WA
E1Dis			Fa, PTime	Fa, WA
E1PF		FSb, Fb		
E2A12	FSb, WA			FSa
E2Dis	FSb	WP		
E2PF		Ext		FSa

Ext			SAP	P1b
FL	P2a, R2b	P2L		P1a, P1b, R1a, R2a
FN		P2b, R1b, R2b		
FSL	R1L, R2L	P2L, R1L, R2L		P1b, R1a, R2a
FSV		PV, R1L		P2b, R1b
FSa	R1a, R2a	P1a, R1a, R2a	R1L, R2L	
FSb	P2a, P2b, R1b, R2b	P2b, R1b, R2B	P1L	R2L
FV		P1b, P2b, R1b, R2b		R2L
Fa	R1a, R2a	P1a, R1a, R2a	PH, SS	P1L, P2L
Fb		P2b, R1b, R2B		R1L, R2L
P1L			WA	PSI0.1, PSI0.5, PSI0.9
P1a		PSI0.1, PSI0.5, WA		
P1b		Trgh	PTime, Stab	
P2L	PSI0.1			WA
P2a	WA	WA		
P2b		SetB, WP		
PH		WA		WP
PSI0.1		R1a	R1a, R2a	R1L
PSI0.5			R2a	
PTemp			R2b	
PTime	R1a, R2a		R1L, R2L	
PV	R2a			
R1L				SetB
R1a		WA		
R1b		SetB, WP		
R2L				SetB
R2a		WA		
R2b		SetB, WP		
SAP		WA		
SS	WA			

[1]**LEGEND** – See Legends for Tables 4.34 and 4.35

Significant relationships were able to be identified between flour, between chapatti, and between flour and chapatti quality traits for the Indian and the Australian hard wheat varieties. The significant correlations present in both of the Indian and the selected Australian hard wheat variety data sets however, were generally different to the significant correlations previously determined. Differences in flour and chapatti quality of the Indian and the Australian hard wheat varieties due to flour blending and the use of a different chapatti making method were likely to have contributed to this outcome.

4.3.4 Conclusion

Chapatti flours of selected Australian hard wheat varieties were blended from two different locations, the Binnu and the Williams trial. The flour quality attributes that were mainly shown to change in value, as a result of blending, were protein content and particle size index. The Indian wheat varieties used in this study, however were the same as those used in the previous research conducted.

The use of a different chapatti making method was shown to produce different quality chapatti for the Indian wheat varieties. Change in the appearance of the chapattis was a notable difference, with smaller darker brown spotting and a lighter chapatti background colour resulting when the second method was used. The second chapatti making method produced acceptable quality chapatti; and chapatti quality was able to be discriminated using the chapatti quality tests. The quality traits of chapatti colour *CIE b**, yellowness, for side one and two; percentage bake loss; the peak force to puncture a chapatti; and percentage surface area of the chapatti puffed; were identified as being important in determining quality differences between the two chapatti making methods. It was generally observed that for some quality traits, such as puffed height, good quality chapatti could be produced, comparable to the first chapatti making method; however for other quality traits like the peak force and work required to puncture a chapatti, poorer chapatti quality was being made.

Differences in chapatti quality between the Indian and the Australian hard wheat varieties were further confirmed. Chapatti quality traits that were important in determining these differences were the sensory assessment score, puffed height, percentage surface area of the chapatti puffed, percentage bake loss and chapatti colour *CIE a**, redness, for side two. Nonetheless, Australian hard wheat variety *Bumper* was shown to have better chapatti quality than the other Australian hard wheat varieties studied; and had some similarities in chapatti quality to Indian wheat variety *C 306* which made good quality chapatti.

Significant relationships were identified between flour, between chapatti, and between flour and chapatti quality traits for the Indian and selected Australian hard wheat varieties. There were both similarities and differences in the significant correlations determined in this study compared to the previous correlation analyses. Differences in flour and chapatti quality of the Indian and the Australian hard wheat varieties due to flour blending and the use of a different chapatti making method were contributing factors.

4.4 Association between wheat protein profiles and chapatti quality

4.4.1 Introduction

The identification of specific proteins that are associated with quality traits, or markers, is an important part of research into the better understanding of wheat quality requirements for end products. The identification of markers can assist in the selection of wheat with desired quality traits at early stages of the breeding program. Many wheat breeding programs currently select for specific combinations of HMW and LMW glutenin subunits for improved quality (Liu et al. 2009). The wheat storage proteins or prolamins consist of monomeric gliadins and polymeric glutenins; which confer properties of viscosity and extensibility, and strength and elasticity to dough (Torbica et al. 2007). The gliadin and glutenin proteins have been intensively studied due to their functionality and influence on the quality of baked wheat foods. The importance of the HMW-GS composition and their effect on pan bread quality has been well established (Liu et al. 2009; Payne 1987). The importance and relationship of specific protein components to chapatti quality however, is not clear. Chapatti, an unleavened bread, is likely to have different protein quality requirements to baked leavened wheat foods.

Research was conducted using matrix assisted laser desorption/ ionisation time of flight mass spectrometry (MALDI TOF MS) to further elucidate and understand the role wheat proteins have on chapatti quality. MALDI TOF MS has advantages of high throughput, and high mass resolution and accuracy, over alternative protein separation techniques such as SDS PAGE and RP HPLC (Gao et al. 2010). Therefore the technique has greater potential for use as an accurate and rapid screening tool to provide predictions of quality through biochemical markers.

Identifying associations between specific protein components and understanding the relationship of specific proteins to chapatti quality is important due to their potential as biochemical markers for quality traits important for Indian end products and therefore assisting in future initiatives that market Australian wheat to India. If protein quality is important for chapatti quality, then any significant relationships identified could be of assistance in screening of wheat lines for wheat quality improvement breeding programmes. The aim was to characterise the HMW-GS, LMW-GS, gliadins, and water soluble proteins of the Australian and the Indian wheat varieties studied; and investigate their relationship to chapatti quality.

4.4.2 Materials and Methods

Summary of materials and methods

Whole wheat flour from twenty five Australian and six Indian wheat varieties was used for the protein extractions and analysis. The water soluble protein and the gliadin protein analyses used flour samples from both the Binnu trial and the Williams trial, for the Australian wheat varieties; as environmental influences potentially affect expression of these wheat proteins. The analysis of the HMW-GS and LMW-GS from two different locations however was not required as the expression of these proteins is predominantly genetically determined.

The glutenin proteins, HMW-GS and LMW-GS, were extracted from whole wheat flour using 55% 2-propanol and 0.08M tris-HCl containing 1% dithiothreitol; and acetone was used to precipitate proteins. The precipitated HMW and LMW glutenin proteins were dissolved in acetonitrile/ water (v/v 50:50) containing 0.05% v/v trifluoroacetic acid. The water soluble proteins were extracted from whole wheat flour using Mili Q water; and the gliadin proteins were extracted with 30% ethanol, after the water soluble proteins had been removed from the flour. A Voyager DE-PRO TOF mass spectrometer (Applied Biosystems®, Life Technologies Australia Pty. Ltd.) was used to perform MALDI-TOF mass spectrometric analysis; and sinapinic acid was used as the matrix. (Refer to section *3.5 MALDI TOF MS protein quality analysis* for the instrument settings and detailed methodology). The protein extractions and analyses were repeated once for the water soluble and gliadin proteins; and repeated four times for the HMW-GS and LMW-GS proteins.

Data analysis

The mass spectra produced from the Voyager DE-PRO TOF mass spectrometer (Applied Biosystems®, Life Technologies Australia Pty. Ltd.) were processed using Data Explorer™ software (Applied Biosystems®, Life Technologies Australia Pty.

Ltd.). The mass spectra were processed by applying a baseline correction, followed by noise filter smoothing, with noise removal set at a standard deviation of two. All of the mass spectra were processed according to this procedure.

The HMW-GS proteins were visually interpreted from the peaks present on the mass spectra. Molecular mass data associated with the peaks observed, and published MALDI TOF molecular masses known to correlate with particular HMW-GS, were used to identify the HMW-GS of the Indian and the Australian wheat varieties (Gao et al. 2010; Liu et al. 2009). The findings were further validated by repeated MALDI TOF MS analyses; and confirmation of HMW-GS characterisations of wheat varieties studied with those reported in the literature.

The LMW-GS proteins were also visually interpreted from the peaks shown on the mass spectra. Molecular mass data associated with the peaks observed, and published MALDI TOF molecular masses corresponding with LMW-GS, were used to identify the LMW-GS of the Indian and the Australian wheat varieties (Liu et al. 2010). The LMW-GS were also analysed using proteome software and a dendrogram was produced to display the hierarchical clustering of the Indian and the Australian wheat varieties.

The water soluble proteins and gliadin proteins were qualitatively assessed and compared, but no further analysis was performed at this stage due to the complexity of the mass spectra. Dendrograms however were produced to display the hierarchical clustering of the Australian wheat varieties using the water soluble protein and gliadin protein molecular mass data. A similarity matrix was also constructed to conduct a preliminary assessment of the gliadin protein profile similarity of the Indian and the Australian wheat varieties.

4.4.3 Results and Discussion

HMW-GS proteins

The mass spectra generated from MALDI TOF MS analysis, for each of the Indian and Australian wheat varieties, displayed clear and well defined peaks. Figure 4.14 depicts the mass spectrum generated for Indian wheat variety *HI 1531*, which has good chapatti quality. The subunit expressed at the *Glu-A1* loci was 2*; at the *Glu-B1* loci the expression of subunit 7 was identified; and at the *Glu-D1* loci subunits 2+12 were found to be expressed. Figure 4.15 shows the mass spectrum of one of the Indian wheat varieties known to have poorer chapatti quality, *WH 542*. The subunit expressed at the *Glu-A1* loci was 2*; subunits 7+9 were expressed at the *Glu-B1* loci; and subunits 5+10 were expressed at the *Glu-D1* loci. The main difference between the Indian wheat varieties, which also differed in chapatti quality, appeared to be the expression of subunits at the *Glu-D1* loci. The presence of subunits 2+12 was associated with good chapatti quality in the Indian wheat varieties; *C 306*, *HI 1531* and *PBW 175*. Subunits 5+10 however were associated with poorer quality in the Indian wheat varieties; but subunits 5+10 in the Australian wheat varieties, *Bumper*, may indicate some suitability for chapatti in combination with specific HMW-GS at the *Glu-A1* and *Glu-B1* loci such as 2* and 17+18; respectively; see Table 4.37.

The HMW-GS compositions of the Indian and the Australian wheat varieties are described in Table 4.37. Subunit expression at the *Glu-A1* loci show two of the Indian wheat varieties, which make good quality chapatti, expressing subunit 2* and one wheat variety, *C 306*, express subunit 1. The wheat varieties *K 9107*, good chapatti quality, and *WH 542*, poorer chapatti quality, also express subunit 2* at the *Glu-A1* loci; whereas *PBW 343* has no protein expressed at *Glu-A1* loci. HMW subunit 7, expressed at the *Glu-B1* loci, was present in two of the Indian wheat varieties with good chapatti making quality. The expression of subunit 7 at the *Glu-B1* loci in Australian wheat varieties is very low; only approximately 0.9% of Australian wheat varieties express this subunit at the *Glu-B1* loci (Ma 2009).

Figure 4.14. MALDI-TOF mass spectrum of the HMW-GS composition of Indian wheat variety *HI 1531*.

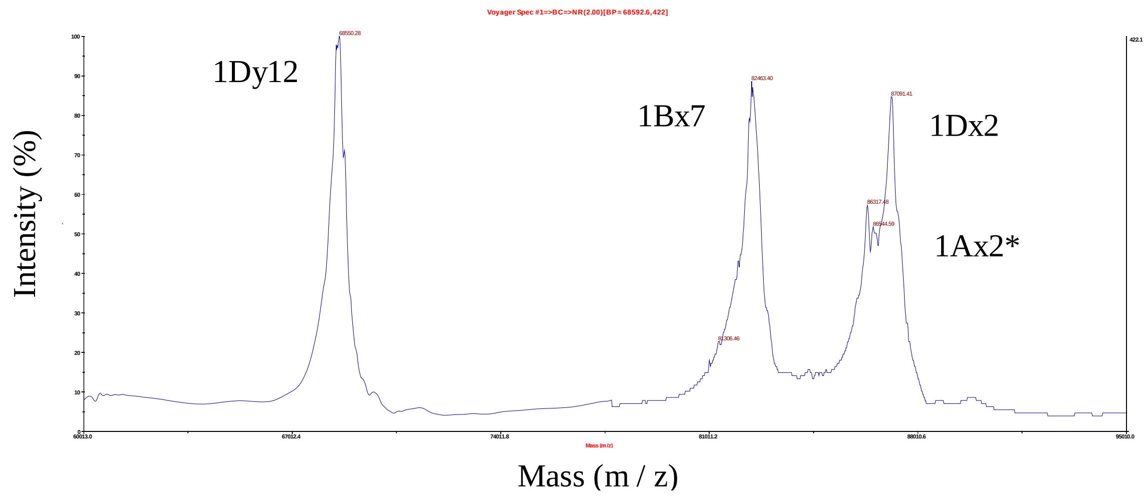
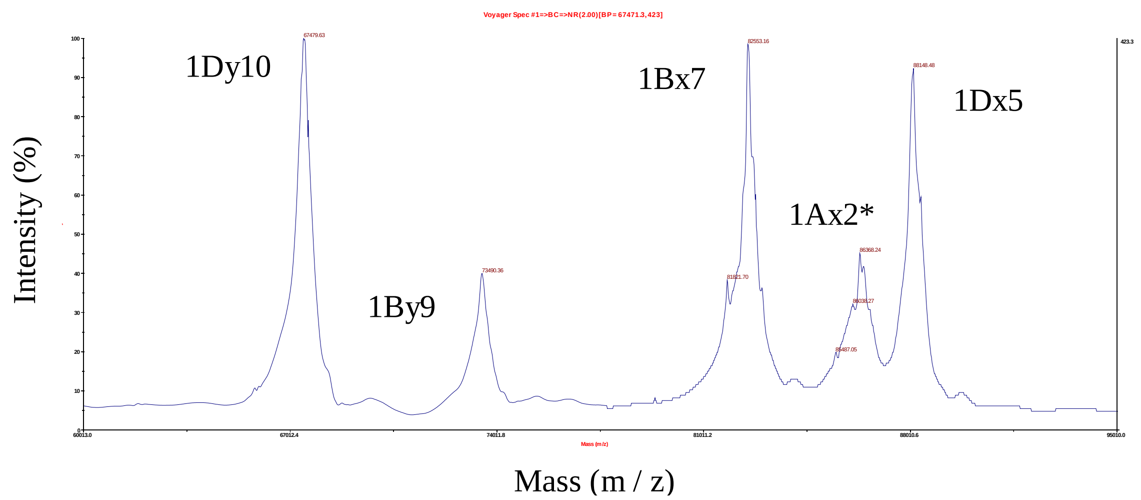


Figure 4.15. MALDI-TOF mass spectrum of the HMW-GS composition of Indian wheat variety *WH 542*.



The subunits expressed at the *Glu-D1* loci were either 2+12 or 5+10; or a combination of both for heterozygous wheat varieties. Three Indian wheat varieties with good chapatti making quality, *C 306*, *HI 1531* and *PBW 175*, expressed subunits 2+12 at the *Glu-D1* loci. The Australian wheat varieties which had higher sensory assessment scores (see Table 4.30) *Mace* and *Tammarin Rock* were also shown to express subunits 2+12 at the *Glu-D1* loci. In addition, *Magenta* was found to be heterozygous, expressing subunits 2+12 and 5+10 at the *Glu-D1* loci, which was the same as Indian wheat variety *K 9107* reported to make good quality chapatti. Conversely, the Indian wheat varieties with average and poorer chapatti quality, *PBW 343* and *WH 542*, respectively, expressed subunits 5+10 at the *Glu-D1* loci; see Table 4.37. The HMW-GS are usually associated with dough strength; and subunits 5+10 have been widely reported to have positive associations with good pan bread making quality (Shewry, Tatham, and Lazzeri 1997). The genotyping results imply that there may be a correlation between HMW-GS at the *Glu-D1* loci and chapatti quality; thus it appears that dough strength and elasticity may not be critical for chapatti making. It is however more likely that specific combinations of HMW-GS is needed for chapatti quality and further investigation into the possible marker combinations is needed.

Table 4.37. Allelic variation at *Glu-A1*, *Glu-B1* and *Glu-D1* loci of the Indian and the Australian wheat varieties as identified by MALDI TOF MS.

Wheat Variety	Type	*Glu-A1*	*Glu-B1*	*Glu-D1*
C 306	Indian	1	7	2 + 12
HI 1531	Indian	2*	7	2 + 12
PBW 175	Indian	2*	7 + 8	2 + 12
K 9107	Indian	2*	17 + 18	2 + 12 & 5 + 10
PBW 343	Indian	Null	13	5 + 10
WH 542	Indian	2*	7+ 9	5 + 10
Bumper	Australian Hard	2*	17 + 18	5 + 10
Carnamah	Australian Hard	2*	7 + 9	2 + 12
Cascades	Australian Hard	1	7 + 9	5 + 10
Correll	Australian Hard	1	7 + 8	5 + 10
EGA Wentworth	Australian Hard	1	7 + 8	2 + 12
EGABonnie Rock	Australian Hard	1	17 + 18	2 + 12
Espada	Australian Hard	1	7 + 8	5 + 10
Fang	Australian Hard	1	7 + 9	2 + 12
Gladius	Australian Hard	Null	7 + 8	5 + 10
Guardian	Australian Hard	1	7 + 8	5 + 10
Kennedy	Australian Hard	1	17 + 18	5 + 10
Mace	Australian Hard	1	7oe + 8	2 + 12
Magenta	Australian Hard	2*	7oe + 8	2 + 12 & 5 + 10
Peake	Australian Hard	2*	7 + 8	2 + 12
Tammarin Rock	Australian Hard	2*	7 + 9	2 + 12
Westonia	Australian Hard	2*	17 + 18	2 + 12
Wyalkatchem	Australian Hard	1	7oe + 8	2 + 12
Yitpi	Australian Hard	1*	7 + 8	5 + 10
Young	Australian Hard	1	7 + 8	5 + 10
Zippy	Australian Hard	2*	13 + 16	2 + 12
Arrino	Australian Soft	2*	17 + 18	2 + 12
Binnu	Australian Soft	1	17 + 18	2 + 12
Calingiri	Australian Soft	1	13 + 16	2 + 12
Fortune	Australian Soft	2*	13 + 16	2 + 12
Yandanooka	Australian Soft	2*	13 + 16	2 + 12

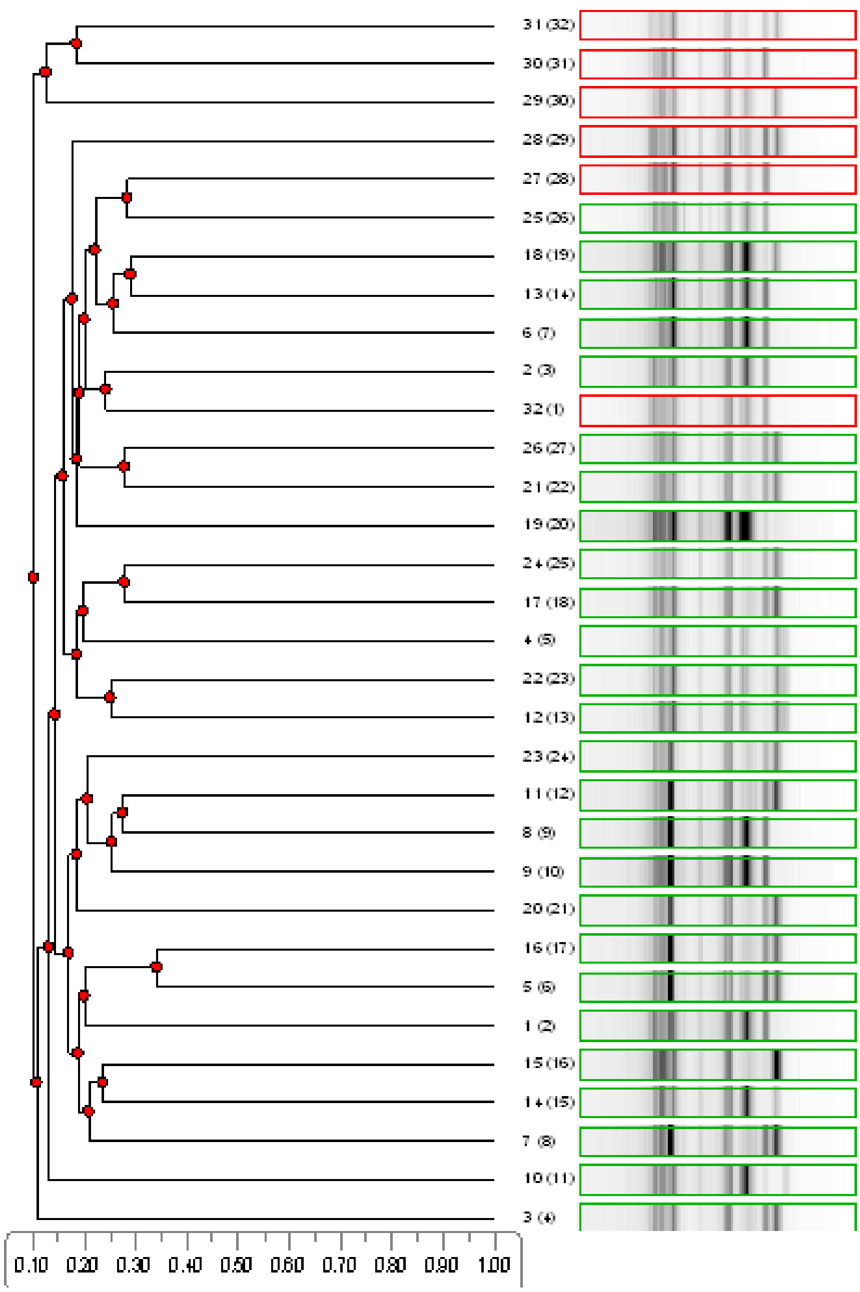
LEGEND – Indian wheat varieties highlighted in BLUE make good chapatti quality; Australian wheat varieties highlighted in GREEN made better quality chapatti using the second chapatti making method

LMW-GS proteins

A dendrogram of the LMW-GS for the Indian and the Australian wheat varieties was generated and is shown as Figure 4.16. LMW-GS are key contributing proteins for dough extensibility. Chapatti quality was shown to correlate well to the LMW-GS variation, indicating that dough extensibility may be an important factor for chapatti quality. Good quality chapatti may need low or a specific range of dough extensibility and this needs to be further investigated.

Figure 4.16. Dendrogram of LMW-GS profiles of the Indian and the Australian wheat varieties.[1]

[1] **LEGEND - Red = Indian wheat variety | Green = Australian wheat variety**

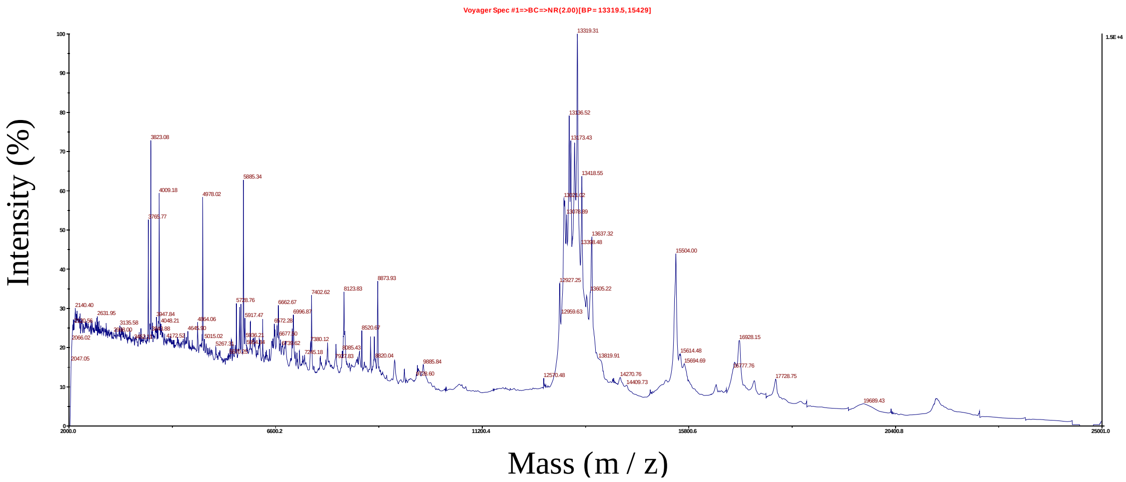


Wheat Variety
C 306
WH 542
K 9107
HI 1531
PBW 343
Zippy
Peake
Gladius
Cascades
Binnu
PBW 175
Wyalkatchem (c)
Wyalkatchem
Tammarin Rock
Young
Magenta
Calingiri
Yandanooka
Fortune
Yitpi
Fang
EGA Wentworth
EGABonnie Rock
Westonia
Mace
Carnamah
Arrino
Kennedy
Guardian
Correll
Espada
Bumper

Water soluble proteins

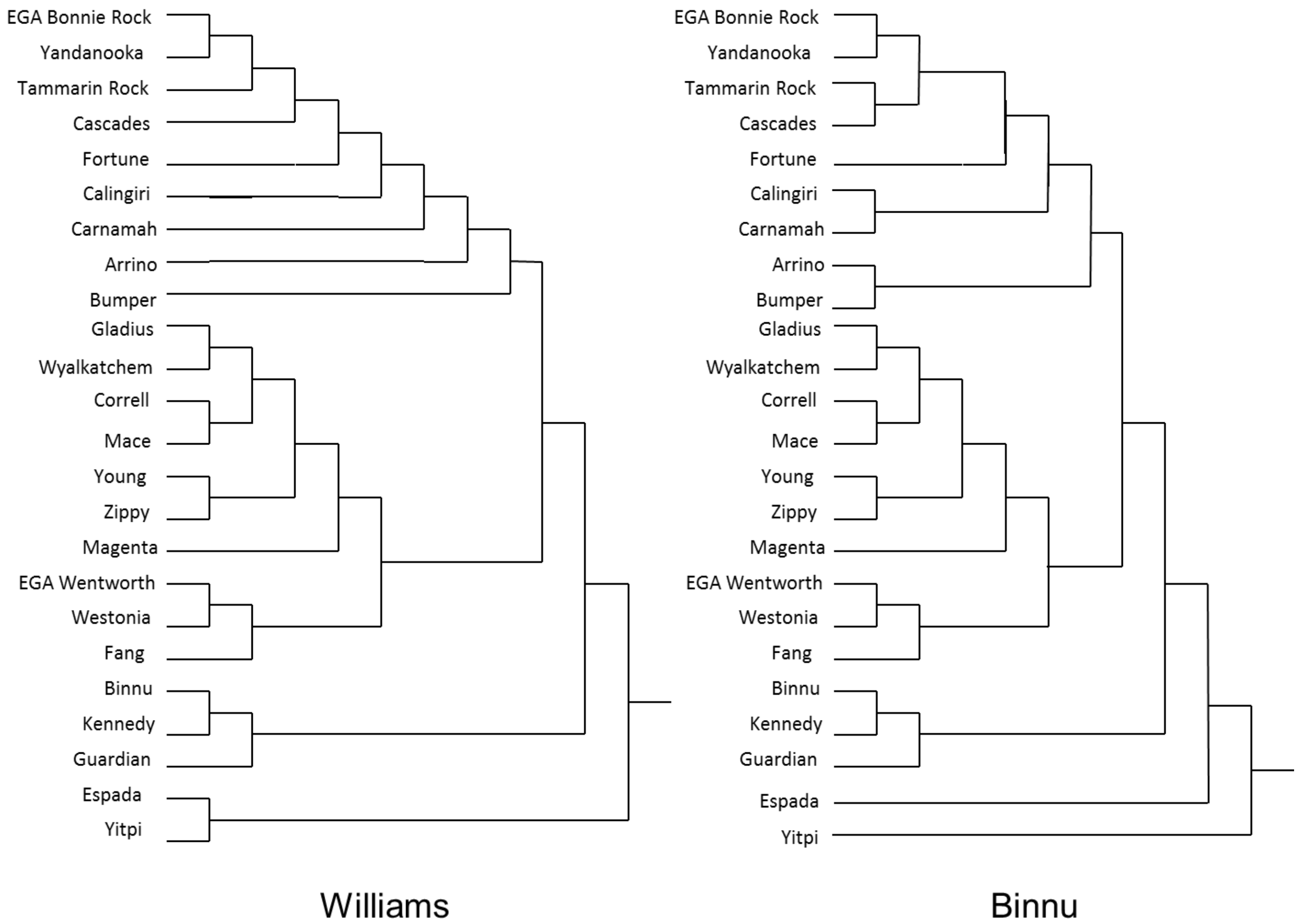
The water soluble proteins, or albumins, were extracted from the Indian and the Australian wheat varieties and separated by MALDI TOF MS. Figure 4.17 is an example of the type of mass spectrum produced for the Australian wheat varieties and shows the water soluble proteins of wheat variety, *Bumper*. The water soluble, albumin, proteins are numerous and have been identified as belonging to families of heat shock proteins, α and β amylases, trypsin inhibitors, UDP-glucose pyrophosphorylases, peroxidases and thioredoxins (Merlino et al. 2009; Dupont and Altenbach 2003). Further analysis would be required to identify the water soluble proteins of the Indian and the Australian wheat varieties and gain further understanding of their importance, if any, to chapatti quality.

Figure 4.17. MALDI TOF mass spectrum of the water soluble proteins extracted from Australian wheat variety *Bumper* from the Williams trial.



Dendrograms were also constructed and display the hierarchical clustering of the Australian wheat varieties; see Figure 4.18. The hierarchical clustering of the Australian wheat varieties from the two different locations, the Binnu trial and the Williams trial, was shown to be the same. There was however minor differences between trials for some of the specific relationships between wheat varieties based on their water soluble protein profile. In this study, environmental influences appear to have had minimal effect on the expression of water soluble proteins of the Australian wheat varieties studied.

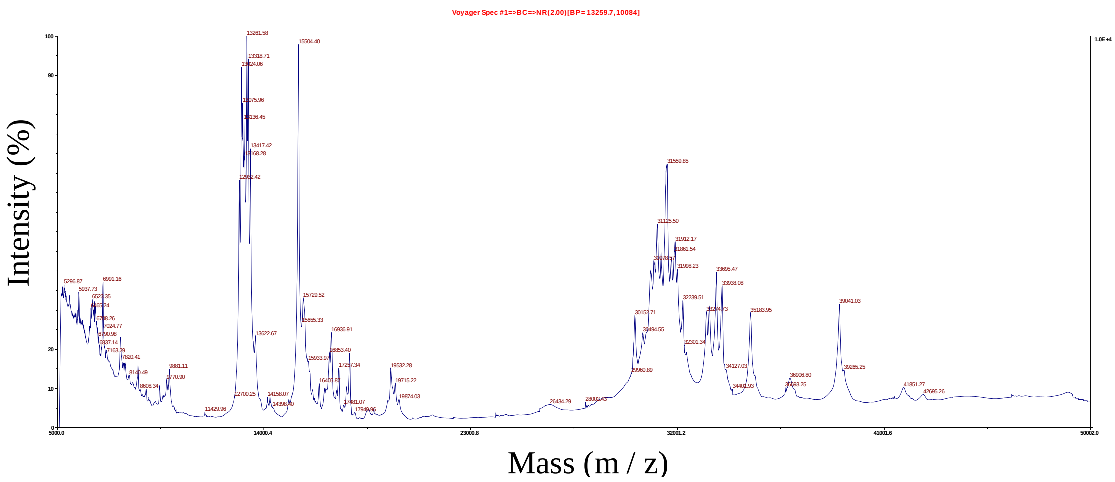
Figure 4.18. Dendrograms of water soluble protein profiles of the Australian wheat varieties from the Binnu trial (Right) and the Williams trial (Left).



Gliadin proteins

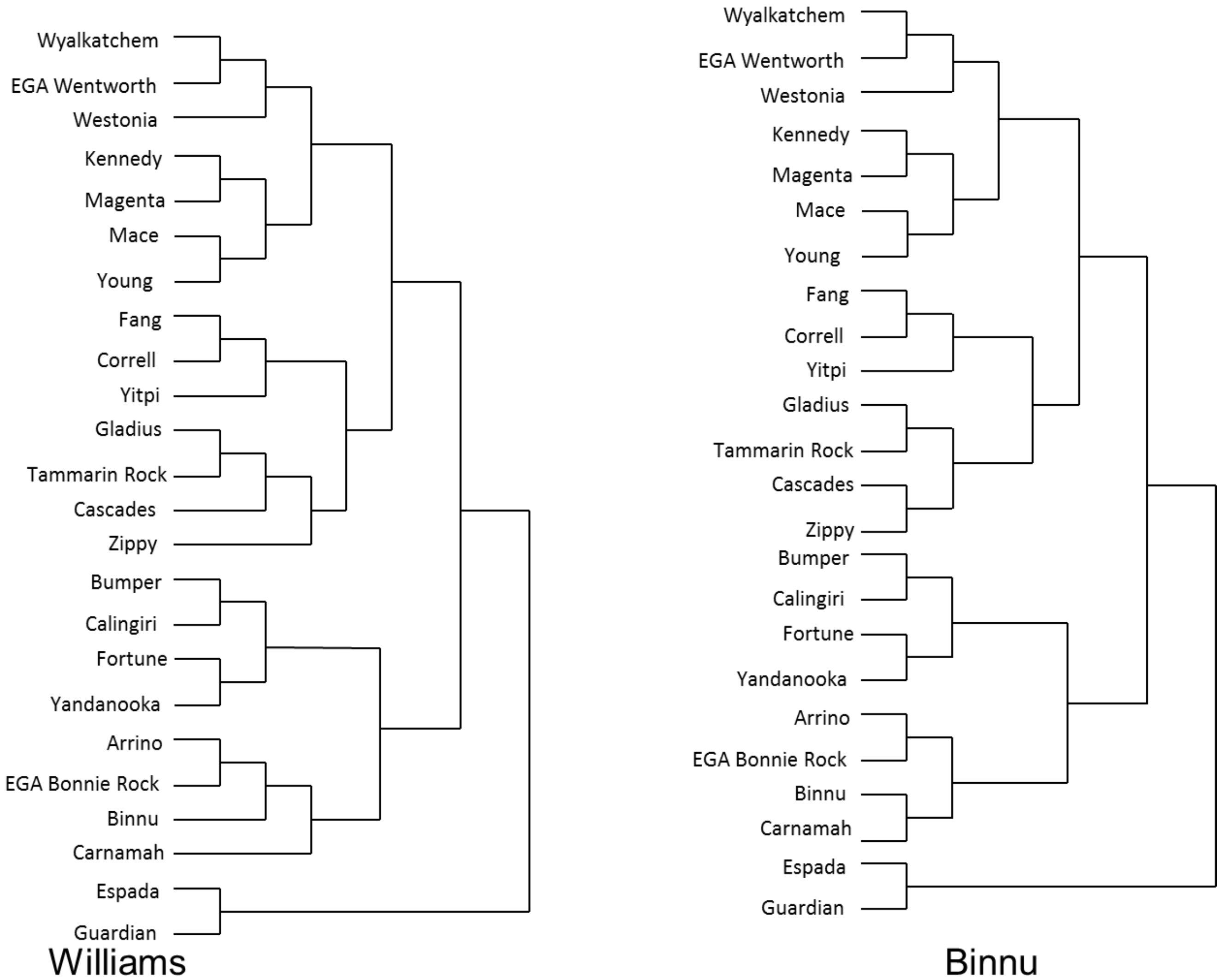
Preliminary qualitative assessment of the mass spectra of the gliadin proteins, for the Indian and the Australian wheat varieties, found that each wheat variety appeared to have a unique gliadin protein composition. Figure 4.19 is an example of the MALDI TOF mass spectrum produced profiling the gliadin proteins of Australian wheat variety, *Bumper*, from the Williams trial. Gliadin protein composition can be used to identify wheat genotypes and therefore different gliadin profiles for the different wheat varieties was expected (Metakovsky and Branlard 1998; Wrigley and Shepherd 1974). Further analysis however would be needed to identify the specific gliadin proteins of the Indian and the Australian wheat varieties, to understand if they play a significant role in determining chapatti quality.

Figure 4.19. MALDI TOF mass spectrum of the gliadin proteins of Australian wheat variety *Bumper* from the Williams trial.



Dendrograms were also constructed using the gliadin protein profiles of the Australian wheat varieties and are shown in Figure 4.20. The hierarchical clustering of the Australian wheat varieties from the two different locations, the Binnu trial and the Williams trial, was shown to be the same. There were minor differences, in the two trials, for some of the specific relationships between wheat varieties based on their gliadin protein profile. The finding in this study supports the use of gliadin protein profiles for wheat genotyping and demonstrated the minimal effect of environmental factors on gliadin protein expression of the Australian wheat varieties.

Figure 4.20. Dendrograms of gliadin protein profiles of the Australian wheat varieties from the Binnu trial (Right) and the Williams trial (Left).



In addition, the gliadin protein profiles of the Indian and the Australian wheat varieties were compared for similarities and a similarity matrix was generated which used the molecular mass data produced from MALDI TOF MS analysis. The similarity matrix of gliadin protein profiles for the Indian and the Australian wheat varieties is shown in Appendix 1.42; wheat varieties with similarity scores equal to or greater than 75 were considered to be similar. The Australian wheat varieties identified as having similar gliadin protein profiles to the Indian wheat varieties are described in Table 4.38. In addition, the Australian wheat varieties which had higher sensory assessment scores in the second chapatti making study, *Bumper*, *Mace*, *Magenta* and *Tammarin Rock*, were also included in Table 4.38.

Nine of the Australian hard wheat varieties, including *Bumper*, *Magenta* and *Tammarin Rock*, were shown to have similar gliadin protein profiles (similarity score equal to or greater than 75) as Indian wheat variety *K 9107*; reported to make good quality chapatti. Furthermore, the Australian wheat varieties, *Bumper*, *Mace*, *Magenta* and *Tammarin Rock*, were shown to have good similarity with the gliadin profile of Indian wheat variety *PBW 175*, also known to make good quality chapatti; see Table 4.38. There were no Indian wheat varieties, of those studied, which were found to be similar to each other and have similarity scores equal to or greater than 75. Despite some similarities, the flour and chapatti quality of the Indian and the Australian wheat varieties was largely found to be different. Differences in gliadin protein profiles were expected and the preliminary results indicated that the gliadin proteins can differentiate wheat varieties and may have relevance to chapatti quality, however further research is needed.

Table 4.38. Indian and Australian wheat varieties with similar gliadin protein profiles as characterised by MALDI TOF MS; and similarity scores of the Australian wheat varieties *Bumper*, *Mace*, *Magenta* and *Tammarin Rock*.

Indian wheat variety	Australian wheat variety from the Binnu trial	Similarity Score (/ 100)	Australian wheat variety from the Williams trial	Similarity Score (/ 100)
HI 1531	Bumper	55	Bumper	66
	Mace	56	Mace	56
	Magenta	66	Magenta	64
	Tammarin Rock	64	Tammarin Rock	65
PBW 175	Bumper	71	Bumper	72
	EGA Wentworth	77	Peake	75
	Mace	65	Mace	73
	Magenta	68	Magenta	72
	Tammarin Rock	74	Tammarin Rock	68
C 306	Bumper	62	Bumper	59
	Mace	55	Mace	55
	Magenta	59	Magenta	61
	Tammarin Rock	61	Tammarin Rock	64
K 9107	Bumper	67	Bumper	80
	Mace	66	Calingiri	79
	Magenta	69	Correll	75
	Tammarin Rock	72	EGABonnie Rock	81
			Mace	70
			Magenta	78
			Tammarin Rock	81
			Westonia	79
			Wyalkatchem	76
			Yitpi	76
PBW 343	Bumper	61	Bumper	69
	Mace	68	Mace	60
	Magenta	60	Magenta	60
	Tammarin Rock	66	Tammarin Rock	66
WH 542	Bumper	64	Bumper	68
	Mace	72	Mace	67
	Magenta	61	Magenta	65
	Tammarin Rock	70	Tammarin Rock	69
			Young	76

4.4.4 Conclusion

The research demonstrated the use of MALDI TOF MS as a rapid and accurate tool for wheat protein profiling and identification. HMW-GS of the Indian and the Australian wheat varieties were characterised. Furthermore, the findings suggested possible correlation between HMW subunits 2+12, expressed at the *Glu-D1* loci, and good chapatti quality. The potential importance of HMW-GS 2+12 however also indicated that dough strength and elasticity may not be critical for chapatti making. Dough which is easy to knead and sheet, without recoil, is needed for good chapatti making; therefore this finding helps support the association of 2+12 with weaker dough strength. Specific combinations of HMW-GS however, which may include subunits 2*, 7+9, 17+18, 7oe+8, and 2+12, are thought to have more importance for chapatti quality.

Chapatti quality was found to correlate well to the LMW-GS variability, which indicated that dough extensibility may be an important factor for chapatti quality. Identification of LMW-GS alleles at *Glu-A3*, *Glu-B3* and *Glu-D3* loci from MALDI TOF mass spectra however needs to be validated to fully explore the relationship between LMW-GS and chapatti quality. Moreover, the gliadin protein profiles of the Indian wheat varieties, with good chapatti quality, and the Australian hard wheat varieties, with acceptable quality chapatti, were found to have similarities and therefore the gliadin proteins may have relevance to chapatti quality.

4.5 Summary

Twenty five Australian wheat varieties, which included both hard wheat and soft noodle wheat types, were selected for study to investigate their abilities to make the Indian wheat food, chapatti. The Australian wheat varieties chosen for the research were determined to have acceptable grain quality; were from two different environmental locations, the Binnu trial and the Williams trial; and represented different Australian wheat grades. Although the grain quality evaluation found the wheat samples grown in W.A, in the 2009 season, to generally have higher screenings and lower protein contents than grade requirements, these factors did not impact on the quality of the grain used in this research. All grain samples subsequently used, had plump well filled grains, greater than 2.0 mm in diameter, and the lower protein contents were more suited to chapatti making. Six Indian wheat varieties, milled for this project, were from India and used as benchmarks to compare flour and chapatti quality. Two of these Indian wheat varieties, *HI 1531* and *PBW 175,* were known to make good quality chapatti and were therefore used as gold standards.

The overall flour quality of the Indian and the Australian wheat varieties was found to be different, and the need for hard wheat types for chapatti making was further confirmed. The Indian wheat varieties, and therefore the chapatti flours considered to make better quality chapatti, had higher ash content, damaged starch content and water absorption; but lower dough extension and particle size index values; in comparison to the Australian hard wheat varieties. The flour quality traits which were also important for chapatti making included having medium hard grain, which may not be common for Australian wheat varieties; and protein contents in the range of 10 to 12% with possibly the presence of HMW-GS 2+12 at the *Glu-D1* loci, as dough strength and elasticity do not appear to be important for chapatti making. The Indian chapatti flours were also shown to be lighter and less red and yellow than the Australian hard wheat varieties, which may be related to their smaller particle size and genetic differences from different wheat breeding programs.

The assessment of chapatti quality of the Indian and the Australian wheat varieties revealed differences in chapatti quality however there were also some similarities, which indicated the chapatti making process was not strongly influenced by all of the flour quality traits determined to be important for distinguishing the Indian and the Australian hard wheat varieties. All of the chapatti quality traits measured were shown to be important for determining chapatti quality. Good quality chapatti needs higher values for puffed height and for the chapatti to puff fully across the entire surface area of the chapatti. The texture needs to be soft and not tough and this can be measured by peak force and the work required to tear and puncture chapatti samples; the chapattis should also have medium extensibility. Baked chapatti colour should be light and creamy brown; however golden brown may also be acceptable to some consumers. Australian hard wheat variety *Bumper* was identified as having better chapatti quality than the other Australian hard wheat varieties, and closer to the chapatti quality of the Indian wheat varieties studied. The Indian wheat varieties reported to have good quality chapatti, *HI 1531*, *PBW 175* and *C 306*, were also found to make good quality chapatti in this research.

Lastly, the use of two different chapatti making methods determined that different quality chapatti was produced for the Indian and the Australian wheat varieties. Furthermore, the research identified the need for an optimised chapatti making method for the particular type of wheat varieties being made into chapatti. A different chapatti making method, similar to the first method used, may assist in producing better quality chapatti for the Australian hard wheat varieties.

5.0 CONCLUSION AND FUTURE DIRECTIONS

Australian wheat varieties were harvested from five trials in W.A. and two trials, Binnu and Williams, selected for further study. From the two trials, commercially available Australian wheat varieties were selected; and they included twenty Australian hard wheat varieties and five Australian soft noodle wheat varieties. The Australian wheat varieties represented a range of different wheat qualities to assist in the study of chapatti quality. Six Indian wheat varieties were also studied and used as benchmarks. Two of the Indian wheat varieties, *HI 1531* and *PBW 175*, were used as gold standards as they both made, and were reported to make good quality chapatti. The grain samples were stone milled producing chapatti flours of high extraction (100% minus coarse bran particles) and the quality of the flours and chapattis determined.

Flour quality and chapatti quality was significantly correlated but the Australian hard wheat varieties from the Binnu and the Williams trials were not closely related to the Indian wheat varieties. The measurement of flour protein content, damaged starch content, water absorption, flour colour and dough extension were predictors of chapatti quality. Flour quality requirements of high damaged starch and high water absorption were found to be important; and confirmed the previously reported wheat quality requirements for chapatti. Higher damaged starch facilitated greater water absorption of the flour, which is important for chapatti making to achieve dough of suitable consistency for kneading and sheeting; and water for steam leavening of the chapatti to create two characteristic layers during baking.

The flour and chapatti quality comparisons with the Indian wheat varieties showed that hard wheat types are needed for chapatti making, and not soft wheat types. The flour quality requirements for chapatti, like high damaged starch content and high water absorption, are some of the quality characteristics hard wheat types provide in comparison to soft wheats. The use of medium hard grain however has been described for chapatti making and evidence of this was demonstrated by the finer particle size of the Indian wheat varieties in comparison to the Australian hard wheat varieties. The difference in particle size of the chapatti flours may have influence on chapatti quality and further investigation of the effect of grain hardness and particle size on chapatti quality should be conducted. The grain hardness of the Indian hard wheat varieties appears to be unique, and not as hard, as the Australian hard wheat varieties studied.

Protein content of 10 to 12% and low dough extension properties were also identified as being important for chapatti making. Dough which is easy to knead and sheet without recoil is desirable for chapatti making; therefore dough should not be overly strong or elastic. The Indian wheat varieties were observed to have these attributes but the Australian hard wheat varieties had greater recoil and elastic properties when sheeting. In addition, HMW-GS 2+12 located at the *Glu-D1* loci were determined to be present in the Indian wheat varieties known to make good quality chapatti and therefore may have potential as a preliminary screening quality trait. Specific combinations of HMW-GS however are thought to have more importance for chapatti quality and in relation to other proteins. The Australian wheat varieties shown to make acceptable quality chapatti had some similarities in HMW-GS and gliadin protein composition to the Indian wheat varieties which make good quality chapatti. Further research specifically investigating protein profiles and their relationship with chapatti quality needs to be conducted to explore these initial findings.

Further knowledge has been gained regarding the wheat quality requirements for chapatti however further investigation into the findings from this research would be beneficial. Greater investigation of the effects of protein quality differences and starch quality differences on chapatti quality would generate further understanding of the importance of these attributes for chapatti quality. In this research, the study of the chapatti making abilities of a range of Australian wheat varieties, which had varying wheat qualities, emphasised the importance of understanding which quality attributes define high quality chapatti. The findings from this research will help in screening wheat samples for future studies.

Two chapatti making methods were trialled, based on Indian chapatti making methods. The Australian wheat varieties generally made better quality chapattis with the first laboratory chapatti making method; however acceptable quality chapattis were also made using the second method. Australian hard wheat varieties *Bumper*, *Correll, Espada, Fang, Gladius* and *Magenta* were found to have similar textural properties, softer texture, to the Indian wheat varieties *HI 1531* and *PBW 175* as assessed by the first method. In addition, *Bumper* had similar chapatti colour to the Indian wheat varieties, in particular *PBW 175*, and the lowest extensibility of the Australian wheat varieties studied. The Australian hard wheat varieties *Bumper*, *Mace*, *Magenta* and *Tammarin Rock* had the highest sensory assessment scores when made using the second chapatti making method and had acceptable quality chapatti. The development and use of two different chapatti making methods highlighted the effect the chapatti making method has on different chapatti quality traits. Thus there is potential for the future development of an optimised chapatti making method for Australian wheat.

Overall the Australian wheat varieties were not able to make chapatti of the same quality as the Indian wheat varieties, which made good quality chapatti. The ability of the Australian wheat varieties however to make acceptable quality chapattis, despite significant differences in flour quality, to the Indian wheat varieties, means they are likely to have the potential to make better quality chapatti with careful selection of flour quality attributes and chapatti making methodology. The objective chapatti quality traits measured, including puffing characteristics, chapatti colour and textural attributes, were all shown to be important for describing chapatti quality. The use of sensory assessment, conducted in the second chapatti making study, was also shown to be valuable for differentiating the chapatti quality of the various wheat varieties.

This research has provided insight into the important flour quality, chapatti quality and chapatti making methodology needed for future selection of wheat for the Indian market.

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