ASSESSMENT OF HECTOLITRE MASS (HLM) EQUIPMENT AND HLM MEASUREMENTS OF OATS

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Declaration

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Abstract

Hectolitre mass (HLM) measurements allow rapid and accurate determination of grain density. HLM devices from different countries (Australia, Canada, France, Germany, South Africa, the United Kingdom and the United States of America [USA]) have been investigated for their effect on the HLM measurements of oats. In addition, the potential of near infrared (NIR) hyperspectral imaging has been evaluated to distinguish between oat samples with different HLM values. Comparing HLM measurements obtained from the respective devices, the USA and the South African devices resulted in significantly ($P<0.05$) lower HLM values compared to the other devices where as the German device resulted in higher values ($P<0.05$) than the other devices. HLM values from all the devices were highly correlated with intra-class correlation (ICC) consistency values of at least 0.90. These high correlations would allow direct replacement of the South African device with any of the other devices. The equipment selected as replacement should ideally be calibrated according to the ISO 7971-3 standard (i.e. the device currently used in Germany).

HLM values significantly ($P<0.05$) increased when oat samples were rubbed before measurements were made, indicating the importance of continuation of this sample preparation step. The investigation on the effect of the operator on HLM determinations showed that the unskilled operator measured HLM values significantly different to those obtained by the skilled operator. This emphasises the importance of training in spite of HLM measurements being a simple procedure.

A poor correlation ($r = 0.18$) was found between protein content and HLM values of oat samples. Moisture content significantly affected the HLM values of oats and results clearly showed a decrease in HLM values with increasing moisture content. Scanning electron microscope (SEM) micrographs revealed that the starch granules became swollen and that they increased in size with an increase in moisture content, resulting in a decrease in HLM. NIR hyperspectral imaging offers the testing of individual grains non-destructively. This is often required by plant breeders because they subsequently need to plant selected grains. NIR offers this option to plant breeders. NIR hyperspectral imaging, which combines NIR spectroscopy with digital imaging, was used to distinguish between six oat samples with varying HLM values. NIR spectroscopic differences were observed between the images of the two samples with the highest and lowest HLM values (60.2 and 49.35 $kg.hL^{-1}$). Less distinct differences were observed in the NIR hyperspectral images of two samples differing by less than 2.0 $kg.hL^{-1}$.

Although mixed oat samples were used, these preliminary results established the possible use of NIR hyperspectral imaging in evaluating oat samples from breeding trials. The use of this technique could also be extended to evaluation of other quality characteristics of oats.

Uittreksel

Hektolitermassa- (HLM-)metings maak snelle en akkurate bepaling van korreldigtheid moontlik. HLM-toestelle van verskillende lande (Australië, Kanada, Frankryk, Duitsland, Suid-Afrika, die Verenigde Koninkryk en die Verenigde State van Amerika) is ondersoek vir hulle uitwerking op die HLM-metings van hawer. Daarby is die potensiaal van naby-infrarooi- (NIR-)hiperspektrale beelding geëvalueer om tussen hawermonsters met verskillende HLM-waardes te onderskei. Tydens vergelyking van HLM-metings verkry van die onderskeie toestelle, het die Amerikaanse en die Suid-Afrikaanse toestelle beduidend (P<0.05) laer HLM-waardes opgelewer in vergelyking met die ander toestelle terwyl die Duitse toestel hoër waardes (P<0.05) as die ander toestelle getoon het. Daar was hoë korrelasies tussen die HLM waardes verkry van die apparate met intraklaskorrelasie (IKK) konsekwentheidwaardes van ten minste 0.90. Hierdie hoë korrelasies sou direkte vervanging van die Suid-Afrikaanse toestel met enige van die ander toestelle moontlik maak. Die toerusting gekies as vervanging sou ideaal gesproke in ooreenstemming met die ISO 7971-3 standaard gekalibreer kon word (bv. die toestel wat tans in Duitsland gebruik word).

HLM-waardes het beduidend (P<0.05) verhoog toe hawermonsters gevryf is voor metings gemaak is, wat dui op die belang van verlengde gebruik van hierdie stap tydens die voorbereiding van monsters. Die ondersoek na die uitwerking van die operateur op HLM-bepalings het getoon dat die onervare operateur HLM-waardes beduidend verskillend gemeet het teenoor dié verkry deur die ervare operateur. Dit beklemtoon die belang van opleiding ten spyte daarvan dat HLM-metings ’n eenvoudige prosedure is.

’n Swak korrelasie (r = 0.18) is aangetref tussen proteïeninhoud en HLM-waardes van hawermonsters. Voginhoud het die HLM-waardes van hawer beduidend beïnvloed en resultate het duidelik ’n styging in HLM-waardes met verhoging van die voginhoud getoon. Aftaselektronmikroskoop- (AEM-)mikrobeelde het aangedui dat die styselgranules swel en in grootte toeneem met verhoging van die voginhoud, wat aanleiding gee tot ’n verlaging in HLM. NIR-hiperspektrale beelding maak die toets van individuele korrels op niedestruktiewe wyse moontlik. Dit word dikwels deur plantkwekers vereis aangesien hulle na toetsing uitgesoekte korrels moet plant. Naby-infrarooi bied hierdie opsie aan plantkwekers. NIR-hiperspektrale beelding, wat NIR-spektroskopie met digitale beelding kombineer, is gebruik om te onderskei tussen ses hawermonsters met wisselende HLM-waardes. NIR-spektroskopiese verskille tussen

die beelde van die twee monsters met die hoogste en laagste HLM-waardes (60.2 en 49.35 kg.hL^{-1}) is waargeneem. Minder duidelike verskille is in die NIR-hiperspektrale beelde van twee monsters wat met minder as 2.0 kg.hL^{-1} verskil het, waargeneem.

Alhoewel gemengde hawermonsters gebruik is, het hierdie voorlopige resultate die moontlike gebruik van NIR-hiperspektrale beelding by die evaluering van hawermonsters van kweekproewe vasgestel. Die gebruik van hierdie tegniek sou ook uitgebrei kon word tot die evaluering van ander kwaliteitseienskappe van hawer.

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List of abbreviations

AACC	American Association of Cereal Chemists
Anon.	Anonymous
ARC	Agricultural Research Council
ca.	circa (about)
e.g.	*exempli gratia* (for example)
et al.	*et alibi* (and elsewhere)
Fig.	Figure
HLM	hectolitre mass
i.e.	*id est* (that is)
ICC	intra-class correlation
IKK	intra-klass korrelasie
$kg.hL^{-1}$	kilograms per hectolitre
$lb.bu^{-1}$	pounds per bushel
NIR	near infrared
PCA	principal component analysis
RANOVA	repeated analysis of variance
RTE	ready-to-eat
SA	South Africa
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture

Chapter 1

Introduction

Chapter 1

Introduction

Hectolitre mass (HLM) is an internationally accepted industry standard for grading cereal grains. It is the mass of grain that fits into a specified volume (Doehlert *et al.*, 2006) and it is reported in kilograms per hectolitre (kg.hL^{-1}). HLM is referred to in some countries as bushel weight, specific weight, test weight or hectolitre weight (Hook, 1984). It is directly related to the density and soundness of the grain. HLM is often a topic of conversation and controversy among grain growers as it affects market price. It is of particular concern in years when the growth and maturity of cereal grains had been challenged. HLM determination has a long standing use in the grain industry as a receival and trading standard for cereals due to HLM being related to grain quality. Thus barley with higher HLM values are required by maltsters and feedlotters (Fox *et al.*, 2007). A higher HLM in oats is related to improved dehulling efficiency and production of maximum sized oat flakes (Doehlert & Wiessenborn, 2007). Also, maize with low HLM has been shown to have lower percentage of hard endosperm and therefore produces lower yields when milled (Rutledge, 1978; Engelbrecht, 2007). In addition, grain is transported in ‘volumetric holds’ such as trucks, rail wagons, containers and ships’ holds, yet the transport cost is charged on a ‘by-weight’ basis (Fox *et al.*, 2007). The HLM of a sample thus provides information for bulk grain handlers during stock management and assists marketers to calculate the cost of supplying grain and its value, depending on demand.

For commercial purposes, oat quality is frequently graded based on HLM, presence of foreign matter and the physical appearance of the grain (Doehlert, 2002). More detailed quality analyses may include evaluation of percentage groat, kernel size and uniformity, and groat composition. HLM is one of the oldest specifications used in oat grading and serves as a guide for a combination of characteristics. This property depends not only on the intrinsic quality of the grain, but also on the grain’s moisture content; the capacity, shape and dimensions of the receptacle used to measure HLM; as well as the way in which the receptacle is filled (Anon., 1974).

HLM of oats is reported to be affected by kernel and groat size; groat density; hull thickness and length; groat percentage (Doehlert *et al.*, 2006); as well as the presence of awns, diseases and tertiary kernels (Murphy *et al.*, 1940; Atikins, 1943; Forsberg & Reeves, 1992). HLM is also understood to be affected by grain/cultivar type, moisture content and harvest location. Other factors such as kernel shape and surface characteristics also affect packing behaviour and thus the HLM of grains (Lloyd *et al.*, 1999).

HLM constitutes a major role in grain and grading systems (USDA, 1978) and therefore remains important in commercial grain purchases. The market value of oat grain is largely determined by means of bulk density expressed as the HLM of a sample (Doehlert *et al.*, 2006). The ease and speed of HLM measurements in the market place has contributed to its wide spread use and acceptance. Plant breeders also use HLM as a deciding characteristic when selecting lines to be released as future cultivars that will yield well and consistently produce high quality oat grain over a wide range of environments.

In South Africa the main grading factor for oats is HLM, expressed in $kg.hL^{-1}$. Large and well-filled kernels or groats are in high demand by the processors and HLM is an indication of this quality characteristic (Anon., 2010a). Because of its wide planting spectrum, adaptability as well as high biomass production, oats are suitable for production in all regions of South Africa (Anon., 2010b). Oat breeding efforts continue to strive to provide improved quality oats for evolving markets. Quality specifications for food and feed applications may eventually require that cultivars be developed with specific target markets. High HLM and high groat percentage will, however, continue to be priorities for all newly bred cultivars.

Two types of HLM equipment are currently being used in different grain producing and exporting countries. South Africa uses a HLM device equipped with a funnel that provides uniform packing in a 500 mL measuring cup (Manley *et al.*, 2009). A wooden scraper is then used to level the grain in the cup. The United States of America (USA) and Canada use devices with packing methods similar to that of the South African device. The second type of HLM equipment is referred to as a chondrometer, which is a cylindrical device. In this case the grain in the top cylinder is separated from the cylinder below by means of a metal blade (cutter). Removing the cutter allows the grain to fill the second cylinder in a controlled manner. Chondrometers are typically used in Australia, United Kingdom, Germany and France.

Recently, different HLM devices have been compared for crops such as wheat (Manley *et al.*, 2009) and maize (Engelbrecht, 2007). In the study of wheat, significantly lower ($P<0.05$) HLM values were observed using the South African device. The device used in Australia resulted in significantly higher ($P<0.05$) HLM values, compared to other devices. Although these devices showed different actual HLM values, they were found to be highly correlated with overall intra-class correlation (ICC) consistency of

0.94. Based on the results obtained in this study (Manley *et al.*, 2009), the grading regulation with reference to HLM measurements of wheat in South Africa have been revised. HLM of wheat can now be determined with any suitable device that had been certified to be compliant to the ISO 7971-3 standard (i.e. the HLM device used in Germany). The South African device can thus now be replaced by any such device. A discompensation of 2.0 kg.hL^{-1} was valid during a limited intermediate period to allow industry to obtain appropriate HLM devices. Currently the general practice in the grain industry is to use the German HLM device to calibrate automatic devices such as those which are part of NIR spectrophotometers or moisture testers. These calibrated devices are then used at grain receiving points to measure HLM.

The study that compared HLM devices using maize (Engelbrecht, 2007) reported different results to those observed in the study of wheat. The devices from Australia and France resulted in significantly higher ($P<0.05$) average HLM values where as the device used in Canada reported significantly lower ($P<0.05$) average HLM values. There were no significant differences ($P>0.05$) in average HLM values obtained from the German, South African, UK, USA. An overall high ICC consistency value of 0.99 was observed (Engelbrecht, 2007). The significant difference in kernel size could have been the reason for the difference in results obtained for wheat and maize, respectively.

With reference to the differences in HLM results seen in previous studies for wheat (Manley *et al.*, 2009) and maize (Engelbrecht, 2007), deductions cannot be made in terms of the results to be obtained from the respective HLM devices when used to determine the HLM of oats. Even though oat is also a small grain, there are physical differences between the grains that can affect HLM determinations. Oat has a different shape to that of wheat. More important though is the fact that oat kernels are covered by glumes which are wrapped securely around the kernel. Wheat, on the other hand has chaff which is easily removed. The South African oat industry is outstanding and contributes about R 44 million (ZAR) towards the gross value of local agricultural production per annum (Anon., 2010b). Assessment of HLM devices using oats would contribute to ensure that the South African oat industry reaches its full potential, both in local and world grain marketing systems. The grading of oats was deregulated in South Africa in 1997. Currently draft grading regulations for oats are used which includes HLM determination (Appendix 1). The outcome of this study will also contribute to reinforce the current status of HLM in South Africa, i.e. possible revision of regulations currently used to grade oats.

Near infrared (NIR) spectroscopy has been used in the cereal industry for efficient quality measurements for many years. The combination of NIR spectroscopy with digital imaging now allows characterisation of cereal grains in a spatial dimension in addition to the spectral dimension. A NIR hyperspectral image represents a set of images measured at different wavelengths that are progressively stacked (Geladi *et al.*, 2004). NIR images are acquired in one of the three ways, namely line scanning imaging (pushbroom imaging), focal plane imaging and point-scan imaging (Burger, 2006; Geladi *et al.*, 2007). Due to the added spatial dimension it allows results for each individual grain to be obtained, even if multiple grains were analysed. In the cereal industry NIR spectroscopy had been used, among others, to distinguish between pre-germinated and non pre-germinated kernels of barley, wheat and sorghum (McGoverin *et al.*, 2011). NIR spectroscopy offers the option to plant breeders to test whole, unground kernels non-destructively; the tested grain can thus subsequently still be propagated. Although conventional NIR spectroscopy has been used to measure HLM of cereal grains, NIR hyperspectral imaging has not been evaluated for measurement of this characteristic. NIR hyperspectral imaging could be a useful technique to South African oat breeders, if HLM is used as a deciding characteristic when selecting future cultivars. NIR hyperspectral imaging has the advantage of analysing a number of grains simultaneously, but results can be obtained for individual kernels. This can be beneficial during early stages of the breeding programs when it would be ideal to analyse kernels non-destructively. This would allow subsequent propagation.

The aim of this study was thus to assess HLM measurements of oats performed using the South African HLM device in comparison to devices used in Australia, Canada, France, Germany, the United Kingdom (UK) and USA. In addition the potential of NIR hyperspectral imaging to characterise oat samples with different HLM values were investigated.

Specific objectives of this study were thus to evaluate:

- HLM equipment used in Australia, Canada, France, Germany, UK and the USA in comparison to the South African device using selected oats samples with a range of HLM values;
- the effect of rubbing of the oats, before the HLM measurement, on the final HLM determinations;
- the effect of the level of skill of operators on HLM determinations;
- the effect of drying and wetting cycles on HLM determinations of oats; and

- the use of NIR hyperspectral imaging to distinguish between oat samples with different HLM values.

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Chapter 2

Literature review

Contents

1. Introduction

Oats (*Avena sativa* L.) are a nutritious, high protein grain crop with important food, feed and value-added applications (Doehlert, 2002). Oats rank sixth in world cereal production, following wheat, maize, rice, barley and sorghum (Stevens *et al.*, 2008). Oat plants are annual grasses belonging to the family Poaceae (formerly known as Gramineae) (Marshal & Sorrells, 1992). Today, oats are an important ingredient in many of the foods we eat. The largest share of oats for human consumption is in hot (cooked) as well as ready-to-eat (RTE) breakfast cereals (Marshal & Sorrells, 1992). Upon receipt of oats as a raw material, whether it is meant for human consumption or for animal feed, it has to undergo quality evaluations.

Improved grain quality benefits the producers as well as the processors. It improves the value of a crop and the value of products manufactured from the grain (Doehlert, 2002). Quality requirements of a specific grain vary depending on the end use of the crop. In oats, millers processing oats for human consumption generally require grain with a high hectolitre mass (HLM), high groat percentage and large groats of uniform size (Doehlert, 2002). Oats with high protein and fat content and lower β-glucan concentrations are usually more desirable for animal feed; because of improved energy content. High groat percentage is also desirable for animal feed because of the low energy content of the largely indigestible hull (Doehlert, 2002).

Amongst many other quality properties, HLM has been generally accepted as one of the most important grading factors. The South African grain grading system also relies heavily on HLM determinations. This literature review focuses on oats as a food crop followed by a review of HLM as a grading factor for cereals in general. With some reference to other grains, factors that can affect HLM measurements especially when measuring oats will be reviewed. This literature will finally include a brief review of near infrared (NIR) spectroscopy, i.e. its background, application with reference to cereals, hyperspectral imaging and image analysis.

2. Application of oats in food systems

2.1 Background

Oats have been described as a grain which “in England is normally given to horses, but in Scotland supports the people” (Karel & Joseph, 2000). Livestock accounted for about 75% of the total consumption of the world’s oat production from 1980 to 1985 (Marshal & Sorrells, 1992). Through the 1980s, 78% of the world’s production was used for livestock feed, 18% for human food, and the remaining 4% for industrial use, seeds and for export (Webster, 1986). More recently, livestock grain feed remained the primary use of the oat crop, accounting for an average of around 74% of the world's total usage in 1991 and 1992 (Stevens *et al.*, 2008). During the same time a revival in the demand for oat based cereal products for human consumption occurred. This coincided with a growing recognition of potential consumer health benefits, i.e. high fiber and cholesterol control properties (Anon., 2002; Anderson *et al.*, 2009).

Over the past ten years South Africa had an average oat production of approximately 39 000 tons per annum, contributing about R 44 million (ZAR) towards the gross value of agricultural production per annum (Anon., 2010b). The average local consumption of oats for processing in the cereal market was approximately 40 000 to 50 000 tons (Anon., 2010a). Because of the overall low quality of oats produced (mainly of a low HLM), a major part of the local oat production is believed to be unsuitable for commercial processing. The requirement of the market is thus filled via imports (Anon., 2010a).

The protein, lipid and carbohydrate levels of oats are superior to that of other cereal grains. These are important factors in human diet and nutrition (Marshal & Sorrells, 1992; Doehlert, 2002; Hermann *et al.*, 2007). Yet oats as a whole grain and oat products represent the smallest fraction of the world's cereal grain consumption (Marshal & Sorrells, 1992). It would seem reasonable to expect that such a highly nutritious and economical protein source would be used increasingly for human consumption both in developed and developing countries.

2.2 Demand for oat products

As expected the demand for foods containing oat products has increased substantially since the health benefits of oats were demonstrated (Anderson & Chen, 1986; Miller *et al.*, 1993). Today, consumers are more health conscious than ever before. They prefer eating food that is high in carbohydrates and fiber but low in sodium and cholesterol. Changing eating habits have inspired the development of new oat products. Instant oatmeal, granola bars, rolled flakes, quick and instant flakes, oat flour, oat bran and RTE breakfast cereals, made with oats, are among the products created to meet the demand of today's consumers. It is well known that these products are relatively quick and easy to prepare.

Oat products are unique in their uses and attributes in comparison to other cereals. They are used with rare exception as whole-grain flake or flour. In contrast to oats, the germ and a significant part of the bran are generally removed from other grains before they are introduced into food systems (Webster, 1986). Oats can also be heat processed to develop the characteristic roasted-oats sensory notes.

2.3 Breakfast cereals

Since oats are not suitable for bread making, due to lack of gluten, it often serve as porridge, flakes or RTE breakfast cereals made from crushed or rolled oats (Marshal & Sorrells, 1992). Consumers are aware of the importance of starting the day with this wholesome breakfast cereal. The major uses of oats are in hot cereals, cold (or RTE) cereals and infant foods. Oatmeal based products are the largest portion of the hot cereal industry (Webster, 1986). Hot cereal products include rolled oats (whole oat flakes) which require five minutes or more to prepare on the stove top and instant oatmeal which is prepared by just adding hot water (Webster, 1986). Instant oatmeal, being prepared in a much shorter time, still offers the convenience of warmth and nourishment typical of hot cereals.

2.4 Functional food properties of oats

2.4.1 Water soluble dietary fiber

Soluble dietary fiber intake has been acknowledged to provide many health benefits. Health claims associated with dietary fiber consumption have been reviewed by (Anderson *et al.*, 2009) and outlined as the ability to reduce risk for developing diseases such as coronary heart disease, stroke, hypertension, diabetes, obesity and certain gastrointestinal disorders. Furthermore, increased consumption of dietary fiber has been associated with improved serum lipid concentrations, lower blood pressure, improved blood glucose control in diabetes, promoting regularity, weight loss and it also appears to improve immune function.

Oats are rich in a wide range of phenolic compounds with proven antioxidant activity in vitro (Masood *et al.*, 2008). As oats are consumed as whole grain, the bran layer which is particularly rich in phenolic compounds is retained. This additional benefit of oats being high in antioxidants led to an even wider appreciation of oats as a human food. Medical research has shown that certain fibrous plant materials in the diet, lower serum-cholesterol concentration (Anderson & Chen, 1986; Anderson *et al.*, 2009). The fiber, however, must be water soluble. Oat bran contains 22% dietary fiber, of which 10.4% is water-soluble β-glucan (Masood *et al.*, 2008). This is in contrast to wheat fiber which is not water-soluble (Masood *et al.*, 2008).

Oat bran is thus rich in water-soluble fiber that is active in lowering blood serum cholesterol. There is no doubt that oat bran and/or whole oats could play a major role in improving health through the diet. Oat contains β-glucan that controls blood glucose and cardiovascular diseases (Anderson & Chen, 1986; Abdellatif *et al.*, 2009; Anderson *et al.*, 2009). Water-soluble fiber in cereals is composed of non-starchy polysaccharides such as β-glucan (Masood *et al.*, 2008) and can form viscous solutions. Increased viscosity in the intestine slows intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine (Masood *et al.*, 2008), making oats a low glycemic index (GI) food (Wood *et al.*, 1990; Granfeldt *et al.*, 2000).

2.4.2 Oats and celiac disease

Celiac disease is an auto-immune hereditary disorder and is caused by a sensitivity to gluten in food (Masood *et al.*, 2008). It can occur in people of all ages starting from mid-infancy. Among celiac patients, a reaction to gluten in food causes damage to villi in the small intestine and prevents effective absorption of nutrients. Malnutrition occurs without these villi; no matter how much food a person consumes, the nutrients from food pass through the gut without being absorbed (malabsorption). This leads to diarrhoea, vitamin and mineral deficiencies, anemia and osteoporosis (Masood *et al.*, 2008). Oats and oat products are known to counteract celiac disease (Masood *et al.*, 2008). The injurious constituent of wheat in patients with celiac disease is α-gliadin in the prolamin fraction of wheat gluten. Oats do not contain gliadin but its counterpart avenin (Barker, 1974). In wheat, rye and barley, prolamins constitute 40-50, 30-50, and 35-45%, respectively of the total proteins, (Masood *et al.*, 2008). However, in the case of oats, prolamins constitute only 10-15% of the total proteins and 60 g of oats are estimated to contain 1.2 g of avenin (Masood *et al.*, 2008).

3. Hectolitre mass (HLM) determination

3.1 Introduction

Hectolitre mass (HLM) is the ratio of the mass of a cereal to the volume it occupies after being poured into the container under well-defined conditions (ISO, 1986). The results are reported in kg.hL^{-1}. It is often also referred to as test weight, specific weight and bushel weight (Hook, 1984). HLM is considered to be one of the most important measures of grain quality and is directly related to the density and soundness of the grain. HLM determination is believed to have been performed as early as the 17th or 18th centuries and to be of British origin (Greenway *et al.*, 1977). It was developed by the grain trade as means of accounting for the varying densities of grain caused by weather and/or cultivation practices (Beuerlein, 2010).

3.2 Advantages of hectolitre mass determination

HLM determination is believed to be faster and easier to carry out than a number of other grain quality measurements, however; it must be done with a high degree of consistency. The popularity of this test is largely due to the ease of the measurement, and its ability to produce a single numerical value (Manley *et al.*, 2009). Other advantages with specific reference to oats have been outlined by Doehlert (2002), i.e. (1) its effectiveness in predicting groat percentage and milling yield; (2) detecting grain damaged by adverse environmental conditions, disease problems or by poor cultural practices; and (3) its ability to provide a definitive value by which the volume required to store or ship a given mass of oat can be calculated. Information obtained from HLM is thus also useful for bulk grain handlers to ensure efficient stock management and it could assist marketers to calculate the cost of supplying grain (Fox *et al.*, 2007). HLM values are useful as grain is transported in 'volumetric holds' such as trucks, rail wagons, containers and ships' holds, but the transport cost is charged on a 'by-weight' basis (Fox *et al.*, 2007).

High HLM values (indicating grain being of good soundness) are desirable (Troccoli & Di Fonzo, 1999) and an indication of grain samples with acceptable visual appeal and high grain density. Low HLM values can occur as a result of various adverse events such as intolerance to weathering (Czarnecki & Evans, 1986), insect damage (Buntin *et al.*, 1992), defoliation (Blum *et al.*, 1991), heat stress (Saadalla *et al.*, 1990), lodging (Laude & Paul, 1956; Weibel & Pendleton, 1964) or delayed harvesting (Pool *et al.*, 1958).

3.3 Hectolitre mass determination of oats

HLM is the most commonly used method to evaluate oat quality (Forsberg & Reeves, 1992) and determine its market value (Doehlert *et al.*, 2006). It is commonly used as an indicator of grain quality and high HLM is generally associated with high grain quality. HLM is thus one of the major indicators of monetary value and an important factor used in grading oats (USDA, 1978). Both the producer and the grain handler prefer oats with a HLM of at least 49 $kg.hL^{-1}$ (Webster, 1986).

In South Africa, the grading of oats was deregulated in 1997 and is now only based on the specifications determined by the buyer (Anon., 2010a). The main quality factor for oats, however, remains its HLM. Large and well-filled groats are in high demand by the processors and HLM is an indication of this quality characteristic (Anon., 2010a). Typical quality requirements for commercial oats, based on HLM, are minimum HLM values of 53 and 48 kg.hL^{-1}, for grades 1 (highest monetary value) and 2, respectively. Feed grade oats should have a minimum HLM value of 38 kg.hL^{-1} (Anon., 2010a).

4. Factors affecting hectolitre mass

4.1 Background

Many factors influence HLM determinations of cereal grains. Some of the most important factors influencing HLM values are plant stresses caused by diseases, insects, soil fertility and/or environmental conditions (e.g. drought, hail, and premature frost) (Rankin, 2009). Anything that impacts the movement of nutrients to the kernel during grain fill or degrades the integrity of the kernel (e.g. ear rots and molds) once it is filled, is believed to lower grain HLM (Rankin, 2009). The physical properties of the kernel, including kernel shape, kernel size, kernel condition, and grain density all influence HLM measurement (Rankin, 2009).

Studies have been done to investigate factors affecting HLM of oat grain. The most important factor is environmental conditions when oats are still growing in the field (Forsberg & Reeves, 1992). Diseases and other stress factors, as well as geographical location, as related to production environment, noticeably influence HLM of oats (Marshal & Sorrells, 1992). HLM of oats is also believed to be affected by groat size, groat density, groat percentage, hull thickness and length, packing efficiency (Doehlert *et al.*, 2006), moisture content and insects (Murphy *et al.*, 1940; Forsberg & Reeves, 1992). The shape of the grain and various seed coat characteristics, including surface texture and cleanliness, particularly influence packing efficiency, which in turn influences HLM measurements (Gaines *et al.*, 1997; Rankin, 2009). The effect of operators and different HLM devices have also been shown to influence the HLM determinations done on wheat and maize (Engelbrecht, 2007).

4.2 Growing environment

The environment in which grain is grown can be determinant in HLM measurements. Two factors that influenced kernel density in soft wheat, i.e. poor grain fill and kernel puffing were shown to affect HLM (Swanson, 1944; Gaines *et al.*, 1997). Poor grain fill causes shriveled kernels. This results in reduction in endosperm, kernel density and HLM values (Gaines *et al.*, 1997). Kernels become puffed when they expand (hydrate) during rain events (Gaines *et al.*, 1997) and do not contract to their original size on drying. When this happens the starch molecules inside the grain are prevented from the natural process of shedding absorbed water molecules that allows the grain to shrink to a normal size. Field rains loosen the bran layer (giving it a puffed appearance) and “disturb” the interior structure of the wheat kernel (Swanson, 1944).

Generally, severe environmental conditions such as high temperatures, drought, or excessive rainfall during grain filling decrease HLM of grains (Shi *et al.*, 1994). An investigation done on six wheat cultivars concluded that elevated temperatures during grain filling can cause kernel shriveling and reduced HLM (Shi *et al.*, 1994). Similar results from other studies showed that shriveled kernels mature earlier than non-shriveled kernels and do not fill completely during endosperm development (Yamazaki & Briggle, 1969a; Yamazaki & Briggle, 1969b; Pena *et al.*, 1982).

An apparent relationship had been found to exist between the environment and HLM of maize (Rankin, 2009). Grain matures and dries naturally in the field. Frequent rain events before harvest may cause the grain to initiate the germination process before harvesting (Rankin, 2009). During germination, oil, starch, and protein are digested to provide nutrients to produce a new seedling. This process leaves small voids inside the grain. Although the grain may again dry in the field, the seed does not always return to its original size and the small voids inside the seed result in a decreased HLM (Rankin, 2009). Endosperm density or texture may be changed by rain events and maize HLM can be reduced by as much as 6.4 kg.hL^{-1} (Rankin, 2009).

The environment in which oats are grown has been found to be a primary determinant of kernel composition. It was reported that environment can greatly affect oat protein content (Long *et al.*, 2006). Nitrogen supply particularly has a strong effect on oat protein and influences kernel composition (Long *et al.*, 2006). A positive correlation was observed between environmental effects and groat percentage (Bartley & Weiss, 1951). In oat spikelets, primary and secondary kernels are believed to have higher groat percentage than tertiary kernels (Bartley & Weiss, 1951). The reason being that tertiary kernels compete with primary and secondary kernels for assimilation (Bartley & Weiss, 1951) thus preventing them from filling properly, resulting in lower density. It appeared that warm spring weather with abundant sunlight were most conducive to improved oat grain yield and quality (Doehlert *et al.*, 2001). Correlation analyses suggested that cooler summer weather without excessive rain during grain fill generated the best oat yields with high quality grain (Doehlert *et al.*, 2001) and high HLM.

4.3 Packing efficiency

Packing efficiency is the percentage volume of the container occupied by the grain (Yamazaki & Briggle, 1969a). The effect of packing efficiency on the HLM determination of wheat has been investigated by a number of researchers (Yamazaki & Briggle, 1969a; Ghaderi *et al.*, 1971; Troccoli & Di Fonzo, 1999). The volume of the wheat grain required to fill a container of specified volume is affected by packing efficiency. Packing efficiency was associated with wheat cultivar as well as grain shape and surface characteristics (Yamazaki & Briggle, 1969a). The shape and surface characteristics of the kernels affected the positioning of each kernel. In addition to the shape and surface characteristic of the grain, another characteristic affecting the positioning of each kernel includes the width-to-length ratio of the grain (Ghaderi *et al.*, 1971). Smooth, clean grain showed higher packing efficiency than uncleaned grain whereas broken, split, flattened or shriveled grain had reduced packing efficiency (Yamazaki & Briggle, 1969a). A number of studies concluded that the most variation in HLM determination of wheat was due to variation in packing efficiency which varied from 53 to 57% (Yamazaki & Briggle, 1969a; Ghaderi *et al.*, 1971; Troccoli & Di Fonzo, 1999).

In the case of barley, grain size was shown to influence HLM values; large plump grains were found to have high HLM values whereas smaller grains resulted in lower HLM values (Fox *et al.*, 2007). However, the HLM of exceptionally large grains decreased due to the physical limitation of large grains filling a small volumetric space efficiently (Fox *et al.*, 2007).

Packing efficiency was shown to also affect the HLM determinations of oats. Oat grain in general were found to have a packing efficiency of 48.7% (Doehlert & McMullen, 2008) with a strong correlation between the width-to-length ratio of the oat grain and HLM values (Symons & Fulcher, 1998). A larger width-to-length ratio of oats represents more spherical kernels that could pack more efficiently (Symons & Fulcher, 1998). These oats may also be denser than longer kernels (Doehlert *et al.*, 2006); resulting in higher HLM values. A number of studies concluded that longer oat kernels resulted in lower HLM values whereas shorter plumper kernels resulted in higher HLM values; largely due to more or less efficient packing (Love, 1914; Zavits, 1927; Barbee, 1935; USDA, 1978; Root, 1979; Forsberg & Reeves, 1992; Doehlert *et al.*, 1999)

It was further stressed that oats with short tight fitting hulls were associated with high packing efficiency and higher HLM values. HLM of oat samples were increased by 20 to 45% by clipping off the tips of oat grains. This is usually done by means of mechanical rubbing and polishing of the oat grain (Cutler, 1940). Frequently, the tips of oats may extend beyond the length of the groats. It has been observed that long kernels (>12 mm), and kernels with “tippy” hulls or awns have more air space between them and pack less well than shorter (10-11 mm) kernels. The tips ‘carry’ empty spaces (Doehlert *et al.*, 2006); thus clipping the tips, rubbing and/or polishing the grain would decrease the empty volumes between the oat grain with improved packing efficiency and increased HLM.

4.4 Kernel density

Oat grain density has been defined as the mean kernel weight divided by the mean volume of the individual kernel (Doehlert & McMullen, 2008). Bulk density is the mass of the grain that fits into a particular volume, expressed as kg.m^{-3} (Doehlert & McMullen, 2008). HLM is a factor closely related to bulk density. The density of the kernel thus influences the HLM of the grain, i.e. wheat that is more dense has a high HLM than less dense oats (Halverson & Zeleny, 1988). Density of grain is normally measured with a pycnometer (Yamazaki & Briggle, 1969a; Chang, 1988; Troccoli & Di Fonzo, 1999; Engelbrecht, 2007). The method uses displacement of a compressed gas to measure an object’s volume such as that of grain. Grain is poured into the cup of the pycnometer, until it overflows, from a funnel suspended above. The excess grain is leveled with the top of the cup and the content is transferred to the pcycnometer to determine the volume where after the grain is weighed. Density (g.mL^{-1}) values are determined from the volume and weight obtained. More recently, a sand displacement method was used to determine the density of oat groats and oat grain, respectively (Doehlert & McMullen, 2008). Oat grain density was found to be 1.3 g.cm^{-3} when using a pcycnometer method (Nelson, 2002). The oat grain densities ranged from 0.96 to 1.03 g.cm^{-3} where as the volumes of individual grains were 31 to 38 mm^{3} (Doehlert & McMullen, 2008). Up to 78% of the variation in oat HLM measurements could be attributed to grain density (Doehlert & McMullen, 2008).

4.5 Groat percentage

The groat is the oat caryopsis that is encased within the oat hull, composed of the lemma and palea (Doehlert *et al.*, 2006). Groat percentage is a measure of the proportion of the whole oat that is recovered as a groat after dehulling (Doehlert *et al.*, 2009). HLM and groat proportion are very important quality characteristics of oat grain. The relationship between HLM and groat percentage had been observed by a number of authors (Stoa *et al.*, 1936; Atikins, 1943; Bartley & Weiss, 1951; Pomeranz *et al.*, 1979; Souza & Sorrells, 1988). The reason for this relationship is that the groat is denser than the oat hull (Doehlert *et al.*, 1999). Analysis of oat grain components indicated groat densities to be *ca.* 1.29 $g.cm^{-3}$ and hull densities were *ca.* 0.69 $g.cm^{-3}$ (Doehlert & McMullen, 2008). Groat proportion was found to account for as much as 34% of variation in HLM (Doehlert *et al.*, 2009).

4.6 Moisture content

Perhaps the most important relationship to understand is that between grain moisture and HLM. As kernel moisture decreases, grain HLM increases (Rankin, 2009). The reason being that as grain dries it also shrinks allowing for more kernels to pack in a test container. This means HLM has an inverse relationship with moisture content. It also follows that high moisture content grains will result in lower HLM. This reduction is mostly due to swelling of the kernels and partly due to the roughening of the bran coat (Lloyd *et al.*, 1999). Swelled kernels have more volume and this reduces the number of grains that will fit in the test container. The effects of change in grain moisture content, by wetting and drying on the HLM had also been observed in winter wheat. The rate of change in the HLM of four winter wheat cultivars with changing grain moisture was greater when the grain was wetted than when it was dried (Pushman, 1975). The HLM of grain which had been dried and returned to its original moisture content was lower than that of original sample (Pushman, 1975). Similar results from another study showed that HLM values significantly ($P<0.05$) decreased from 79.77 to 72.61 $kg.hL^{-1}$ when the moisture content of wheat increased from *ca.* 11 to 18% (Manley *et al.*, 2009).

4.7 Diseases

Crown rust and stem rust are wide-spread and the most important and destructive diseases affecting cultivated oats (Long *et al.*, 2006). It is caused by the fungal pathogen *Puccinia coronate Corda Var. Avenae W. P. Fraser Leadingham*. Environments severely affected by crown rust produced grain with lower HLM values, groat percentage and groat weight in susceptible genotypes (Doehlert *et al.*, 2001). This disease was reported to cause grain yield losses of up to 30% (Endo & Boewe, 1958). Crown rust disease restricts photosynthesis and the ensuing dry matter accumulation in developing groats (Marshal & Sorrells, 1992). Additionally, crown rust disease results in rupture of the host epidermis (Marshal & Sorrells, 1992) and increases water loss by the plant through uncontrolled evaporation. The water loss is also enhanced by impeded root development, further hastening senescence and poor development of the grain (Marshal & Sorrells, 1992). The overall effect of these would be the reduction in yield and grain quality. The effect on grain quality is therefore reflected in reduction of the HLM determinations.

Generally, both stem and crown rust reduce the weight of the kernel by reducing the amount of proteins and carbohydrates. These diseases reduce the carbohydrate of the kernel relative to fiber resulting in light-weight shriveled grains that have increased hull percentage (Murphy, 1953; Sebesta, 1974). A decrease in whole grain protein induced by these diseases has also been reported (Sebesta & Sykora, 1974; Marshal & Sorrells, 1992).

Van Niekerk *et al.* (2001) investigated the effect of leaf rust and crown/stem rust on South African barley and oat cultivars, respectively. *Puccinia hordei* (leaf rust) for barley as well as *Puccinia coronata f. sp. avenae* and *Puccinia graminis f. sp. avenae* (leaf/crown and stem rust) for oats have been found to be important diseases of these respective crops in South Africa. Yield losses of as high as 85% were recorded for leaf (crown) and stem rust while in the case of barley leaf rust, losses of as much as 58% were recorded. Not only were yields influenced by disease but also quality. HLM was reduced by as much as 45% in oats, while kernel plumpness of barley was reduced by up to 65% (Van Niekerk *et al.*, 2001).

4.8 Insects in stored grains

Generally, anything that impacts the movement of nutrients to the kernel during grain fill or degrades the integrity of the kernel once it is filled will lower grain HLM (Rankin, 2009). Weight loss of grains may occur as insects chew or feed on some parts of the grain. Moth larvae may preferentially attack the germ of the grain thus removing a large percentage of the protein and vitamin content, whereas weevils, feeding mainly on the endosperm, will reduce the carbohydrate content (Cuperus *et al.*, 2010). Many pests may eat the bran of cereals reducing vitamin content such as that of thiamin (Cuperus *et al.*, 2010). Primary grain insects are those that are capable of destroying whole, sound grain. Adults have strong jaws that enable them to chew into sound kernels (Cuperus *et al.*, 2010). They deposit eggs on the grain surface and after hatching, the larvae tunnel into the seed and chew out its contents. The overall effect of these would be the reduction in yield and grain quality; reflected in reduction of the HLM. Insects most commonly found in stored oats are the flat grain beetle (*Cryptolestes pusillus)*; the rusty grain beetle (*Cryptolestes ferrugineus*); the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.); the red flour beetle (*Tribolium castaneum)*; the foreign grain beetle,(*Ahasverus advena*); and the hairy fungus beetle (*Typhaea stercorea* L.) (Storey *et al.*, 1983; Throne *et al.*, 2003). Incidence of insects generally increases with increased grain moisture. This increasing number of insects, when present in large numebrs, can in turn result in an increase in moisture content of oats during storage.

Infested oats are oats characterised by the presence of live weevils or other live insects injurious to stored grain (USDA, 2004) and often have lower HLM values. An average HLM of 47.75 kg.hL^{-1} was found in oats infested by 56.4% of one or more live, insect species (Storey *et al.*, 1983).

4.9 Operator

HLM determinations are affected by operators because of the effect of the manner in which the grain is poured into the measuring cups (ISO, 1986). Investigating the effect of different operators on HLM determinations of wheat on two South African devices showed that a significant (P<0.05) operator effect existed between three operators with different levels of skills and experience (Manley *et al.*, 2009). The lowest intra-class correlation (ICC) agreement (0.920) and consistency (0.916) values were observed for the least skilled operator. However, these values did increase with increasing experience and were 0.947 (ICC agreement) and 0.945 for the results obtained by the unskilled operator on the second South African device (Manley *et al.*, 2009). Operators need to receive sufficient training and develop adequate skills in order to be more consistent when performing HLM tests (Engelbrecht, 2007).

When the HLM measurement is performed by the same operator, the repeatability error of the HLM results given by the standard deviation must not exceed ±0.1% for six successive measurements on the same sample of grain (Anon., 1974). If this degree of repeatability is not reached by a skilled operator, the variation might be due to lack of homogeneity in the grain (Anon., 1974). Grain should thus be mixed properly and operators must be consistent in their operating procedures. Throughout the measuring procedure, it is important that the apparatus should not be tapped, knocked or shaken (ISO, 1986). Jolting the instrument during measurements leads to unnecessary compaction of the grain in the measuring cylinder. This results in falsely high HLM values to be measured.

4.10 Hectolitre mass equipment

Different cereal importing and exporting countries tend to have their own HLM devices and associated methods of determination. The two types of devices currently used are the funnel-shaped devices and cylindrical chondrometers. Within the South African grading system a device equipped with a funnel that provides a uniform packing in a 500 mL measuring cup is being used (Manley *et al.*, 2009). The grain flows from the funnel into the measuring cup and excess grains are leveled off with a wooden scraper. The mass of the grain is divided by five to convert it to kg.hL^{-1}. Similar devices are used in Canada, France and USA. The South African device is currently being replaced by the device used in Germany.

Chondrometers are cylindrical devices containing a column in which grains are isolated from the cylinder of known volume underneath by means of a level blade or metal bar (Manley *et al.*, 2009). The blade separates a precise volume of grain (below the blade) from excess grains above the blade (ISO, 1986). This known volume of grain is weighed and the mass converted to kg.hL^{-1} using appropriate conversion charts. These cylindrical type of devices are utilised in Germany, United Kingdom, France and Australia.

Different operating procedures and the different volumes of receiving/measuring cups can influence the HLM of wheat (Manley *et al.*, 2009). The effect of HLM equipment on HLM determinations had been investigated for wheat (Manley *et al.*, 2009) and and maize (Engelbrecht, 2007). Variation between instruments can arise from the manner in which the grain is poured into the measuring container and the manner in which the grain packs into the measuring container (ISO, 1986). Some HLM devices, such as the German device, have a pre-filling metal measure. The pre-filling measure helps to control the manner in which the filling hopper is filled and reduce or eliminate operators' errors. The Australian device does not allow controlled flow of the grain into the receiving cup (Engelbrecht, 2007). The grain falls directly into the measuring cup causing irregular packing which affects HLM measurements (Manley *et al.*, 2009).

Different HLM devices were found to produce different actual HLM values in wheat (Manley *et al.*, 2009) and maize (Engelbrecht, 2007). In the study of wheat, significantly lower ($P<0.05$) average HLM values were measured by the South African device whereas the device used in Australia resulted in significantly higher ($P<0.05$) average HLM values in comparison to the other devices. It was, however, concluded that HLM values obtained from the respective devices were highly correlated with an overall intra-class correlation (ICC) consistency value of 0.94 (Manley *et al.*, 2009).

Compared to the results from the wheat study (Manley *et al.*, 2009), the study that compared HLM devices using maize (Engelbrecht, 2007) showed different results. No significant differences ($P>0.05$) were observed between average HLM values obtained from the German, South African, UK and USA devices. Compared to the other devices, significantly higher average HLM values were reported by ($P<0.05$) the Australian and French devices while the Canadian device showed significantly lower ($P<0.05$) average HLM values. Again, a high overall ICC consistency value of 0.99 was reported thus showing high correlation between HLM values obtained from the respective devices (Engelbrecht, 2007).

5. Near infrared spectroscopy

5.1 Background

Near infrared (NIR) spectroscopy is a vibrational spectroscopic technique based on the interaction between electromagnetic radiation and vibrational modes of covalently bonded molecules (Osborne *et al.*, 1993; Reich, 2005). The NIR spectral region (780 - 2500 nm) lies between the visible and infrared regions in the electromagnetic spectrum (Miller, 2001; Workman & Schenk, 2004). In NIR spectroscopy overtone and combination vibrational modes provide chemical and physical information (Workman & Schenk, 2004; Walsh & Kawano, 2009). When a molecule is exposed to NIR radiation, it becomes excited from the ground state to the second or higher vibrational energy level, resulting in overtones (Osborne *et al.*, 1993; Roux, 2010). Vibrations of C-H, O-H, N-H, and S-H bonds are observed in the NIR region (Pasquini, 2003). The absorption of NIR wavelengths by food constituents such as proteins, fats, carbohydrates and moisture is strong enough to allow accurate measurement (Osborne, 1981).

5.2 Uses and application of NIR spectroscopy

NIR spectroscopy is routinely used as a quality control tool in many industries, e.g. pharmaceuticals (Reich, 2005) and agriculture (Li-chan *et al.*, 2010). It is widely used as an analytical quality control tool for a number of reasons. NIR spectroscopy is (1) rapid (Workman & Schenk, 2004); (2) environmentally friendly by minimising chemical pre-treatments and waste materials (Osborne *et al.*, 1993); and (3) robust and flexible such that unskilled personnel can perform NIR analysis (Osborne *et al.*, 1993; Li-chan *et al.*, 2010).

NIR spectroscopy is widely used to determine the chemical properties of various food stuffs (Li-chan *et al.*, 2010). International bodies, e.g. AACC International (AACC), have prescribed methods using NIR spectroscopy to measure proteins in barley, oats and wheat (AACC, 2009). NIR spectroscopy has also been used to predict other properties of grains. For example in whole brown rice, NIR spectroscopy (1100-2498 nm) was used to determine embryo activity, germination vigour and bulk density (Himmersbach, 2010). NIR spectroscopy was also shown to be able to predict HLM of hard vitreous durum wheat kernels (Williams, 2010), with r^2 values of 0.79 being reported, and to assist in selecting malting barley in breeding programmes (Roux, 2010).

5.3 NIR hyperspectral imaging

In contrast to conventional NIR spectroscopy which can be used to quantify chemical properties, NIR hyperspectral imaging provides, in addition to information describing chemical composition, also the distribution of these compounds within samples (Shahin & Symons, 2008). A NIR hyperspectral image represents a set of images measured at different wavelengths that are progressively stacked (Geladi *et al.*, 2004). NIR images are acquired in one of three ways, namely line scanning imaging (pushbroom imaging), focal plane imaging and point scan imaging (Burger, 2006; Geladi *et al.*, 2007). The line scan method requires relative movement between the camera and sample, unlike in focal plane imaging where both the spectrometer and the sample remain stationery relative to the detector (Geladi *et al.*, 2007). In point scan imaging a spectrum is measured from a single spot on the sample (Burger, 2006) and a new spectrum is obtained from the next spot by repositioning the sample. Line scan imaging is the fastest method followed by focal plane imaging and point scan imaging which is really time-consuming.

In cereal research, NIR hyperspectral imaging has been used to distinguish between pre-germinated and non pre-germinated kernels of barley and sorghum (McGoverin *et al.*, 2011) and for wheat (Koc *et al.*, 2008; Xing *et al.*, 2010; McGoverin *et al.*, 2011); selecting malting barley for breeding programmes (Roux, 2010); investigating conditioning time in hard and soft wheat kernels (Manley *et al.*, 2011); and detecting aflatoxins in single maize kernels (Pearson *et al.*, 2001; Shahin & Symons, 2011).

5.4 Image analysis

Principal components analysis (PCA) is most often used to analyse NIR hyperspectral images (Esbensen & Geladi, 1989; Razifar & Bergstrom, 2007). PCA is used to reduce a data set to a more compact form without losing information (Razifar & Bergstrom, 2007). The ultimate goals of PCA are to recognise patterns, find classes of similar objects within the data, and outlier detection (Wold *et al.*, 1987). Each principal component (PC) calculated in a PCA has a loading, and a score value for each pixel. Scores represent associations between samples whereas the loading describes relationships between variables (i.e. wavelengths) (Geladi *et al.*, 2004). Principal components (PCs) are calculated to explain a decreasing amount of data variance e.g. PC1 = 90%; PC2 = 8%; PC3 = 1%; PC4 = 0.6% and PC% = 0.4% (Burger, 2006; Razifar & Bergstrom, 2007). Original data sets are often pre-processed before data analysis; pre-processing removes certain defects in the spectra, e.g. background noise, instrument drift and light scattering (Ozaki *et al.*, 2007). Typical pre-processing techniques include derivatives, spectral smoothing algorithms, mean-centering and multiplicative scatter correction. Clusters are located and interpreted using a process called brushing (Esbensen & Geladi, 1989). This is achieved by selecting a cluster in the score plot and relating that cluster to a specific area in the score image.

6. Conclusion

The cost of a grain lot is often based on the perceived quality of that lot. Perceived end-use of grains is largely based on HLM and chemical composition of a specific grain, i.e. protein content of wheat and/or of oats. Unfortunately these factors are highly influenced by the growing environment. Thus in practice, commercial end-use of a specific grain depends on its chemical composition and physical condition. HLM is the most common commercial method used to measure grain quality. Despite the fact that it is one of the oldest methods, it is accepted and used in grain marketing systems due to its easiness, fastness and its ability to provide a single numerical value. It can be used as a means of accounting for the varying densities of grain caused by weather and/or production practices.

HLM offers many advantages such as detecting grain damaged by adverse environmental conditions, disease problems or by poor cultivation practices. Commercially, different devices with different operating procedures are being used around the world. This arouses more emphasis being required in evaluating how these different devices correlate with each other in terms of their measured HLM values.

Considering the abovementioned advantages, the capabilities of HLM measurements can be extended to create more uniformity and transparency in world grain marketing systems. The South African grain industry, and particularly the oat sector is no exception.

NIR hyperspectral imaging has been investigated as a rapid technique for food analysis because it requires minimal sample preparation. NIR hyperspectral imaging can be used to investigate physical properties of whole cereal grains and is potentially useful to grain handlers as a method to classify and identify grain. Testing whole, single kernels would be ideal to plant breeders as they can subsequently plant the analysed kernels if required. NIR hyperspectral imaging offers this option to plant breeders.

7. References

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Chapter 3

Assessment of hectolitre mass (HLM) equipment and HLM measurements of oats

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Abstract

Hectolitre mass (HLM) is the mass of a given volume of grain and represents the density of the packed grain. Higher HLM values translate to superior quality and are desirable because they positively influence market grade and price. This study reports results on the HLM measurements of oats and assessment of HLM devices from different grain producing and exporting countries (Australia, Canada, France, Germany, South Africa, the United Kingdom and the United States of America). The South African and the USA devices resulted in HLM values significantly lower ($P<0.05$) where as the German and the Canadian devices measured significantly higher ($P<0.05$) in comparison with the other devices. Even though all devices resulted in different actual HLM values, it has been found that the HLM values from all respective devices were highly correlated with intra-class correlation (ICC) consistencies of higher than 0.90. This indicated the possibility of replacing the South African device with any of the other equipment e.g. the German device. Ideally it should be calibrated according to ISO 7971-3. The results from the rubbing of oats investigation showed a significant increase ($P<0.05$) in HLM values when oat samples were rubbed. The effect of changing the moisture content of oat samples significantly ($P<0.05$) influenced the HLM determination of oats. The importance of training was shown with significant differences in HLM results when measurements were performed by either skilled or unskilled operators.

Introduction

For commercial purposes oat quality is frequently graded based on hectolitre mass (HLM), presence of foreign matter and the physical appearance of the grain (Doehlert, 2002). Other quality analyses may include evaluation of percentage groat, kernel size and uniformity and groat composition (Doehlert, 2002). The ease and speed of HLM measurements has contributed to its wide spread use and acceptance as one of the most important measures of grain quality.

HLM is related to the density and soundness of the grain (Weibel & Pendleton, 1964; Yamazaki & Briggle, 1969) and determined as the mass of grain that fits into a specified volume (Doehlert *et al.*, 2006). The results obtained are then expressed in kilogram per hectolitre ($kg.hL^{-1}$). Although the grading of oats was deregulated in South Africa in 1997 (Anon., 2010), buyers still have specifications on which bases oats are ‘graded’. HLM remains the main ‘grading factor’ and HLM determinations are thus still being performed to ensure the quality of oats complies with the specification of the buyers.

HLM depends not only on the intrinsic quality of the grain, but also on the grain’s moisture content; the capacity, shape and dimensions of the receptacle used to measure its volume; as well as the way in which the receptacle is filled (Anon., 1974). The latter is reported to be affected by kernel and groat size; groat density; hull thickness and length; groat percentage; as well as the presence of awns, diseases and tertiary kernels (Murphy *et al.*, 1940; Atikins, 1943; Forsberg & Reeves, 1992). Other factors such as kernel shape and surface characteristics also affect packing behaviour (Lloyd *et al.*, 1999). In addition HLM is affected by grain/cultivar type and harvest location.

Two types of HLM equipment are currently being used internationally. South Africa (SA) uses a device equipped with a funnel that provides uniform packing in a 500 mL measuring cup (Manley *et al.*, 2009). The United States of America (USA) and Canada use devices with packing methods similar to that of SA. The other type of HLM equipment is cylindrical and is referred to as a chondrometer. This type of device is used in Australia, the United Kingdom (UK), Germany and France.

It has been shown in an earlier study that the different HLM devices produced different actual HLM values when measurements were performed on wheat (Manley *et al.*, 2009) and maize (Engelbrecht, 2007). In the study on wheat, the SA device resulted in HLM values significantly lower ($P<0.05$) and the device used in Australia with values significantly higher compared to the other devices ($P<0.05$). These differences in actual HLM values were confirmed by an overall intra-class correlation (ICC) agreement of 0.52 when mixed cultivars were used. It has, however, been shown that the HLM obtained from the respective devices were highly correlated (overall ICC consistency of 0.94) (Manley *et al.*, 2009). Based on this study, the HLM of a consignment of wheat may be determined with any suitable instrument. This instrument must however comply to, and be calibrated according to, the specifications in ISO 7971-3. Thus any device can be used provided that it is calibrated using the ISO standard. The high correlation between the respective devices resulted in the interim arrangement where a dispensation of 2.0 kg.hL^{-1} was added to any HLM measurements done with a South African device. The SA device must now be replaced with any device calibrated according to the ISO 7971-3 (e.g. the German device).

The results obtained in the study when different HLM devices were compared using maize (Engelbrecht, 2007) were different to those obtained in the study using wheat (Manley *et al.*, 2009). Results obtained from the German, SA, UK and USA devices did not differ significantly ($P>0.05$). The average HLM values obtained from the devices from

Australia and France were significantly (*P*<0.05) higher and those from the Canadian device significantly lower (*P*>0.05). Again the overall ICC agreement was low (0.52) and the ICC consistency high (0.99).

The effect of using these different types of devices when measuring HLM on oats has not been addressed to date. This study will ensure that the South African oat industry reaches its full potential in the world grain marketing systems. The outcome of this study will be useful in determining and reinforcing the current status of HLM in the South African oat grading system. The results will also assist in updating standards currently used to inspect oats and will represent suitable market price. Additionally, this study will assist the commercial oat sector to be aligned within the South African cereal grading system.

The aim of this study was to asses HLM results obtained from the SA device in comparison with the HLM results obtained from those devices by other grain exporting and importing countries (German, Canada, France, Australia, UK and USA) using oat samples selected to cover a range of HLM values. This study also evaluated the effect of operator on HLM determinations of oats and the effect of rubbing of the oats before measurements are performed. In addition the effect off consecutive wetting and drying on HLM determinations of oat samples were determined.

Materials and methods

Oats samples, sample preparation and hectolitre mass devices

Commercial oat samples with a varying range of HLM values were kindly supplied by producers in the Western Cape Province (Kaap Agri; Sentraal-Suid Kooperasie; JH Blanckenberg (Pty) Ltd). The samples were selected to cover a range in HLM values of *ca.* 10.kg.hL$^{-1.}$ The samples were stored at ambient temperature until being used for HLM determination. To prevent insect infestation during that time, particularly the saw-toothed beetle (*Oryzaephilus surinamensis L*.), the samples were regularly sprayed with pyrethroid insecticide. The samples (*ca.* 500 g at a time) were rubbed for 3 min (which is longer than the one min suggested in the draft regulation) in a woven cloth sack before the HLM measurements were performed. The longer rubbing time was introduced in this study to ensure efficient clipping of the hull tips, removal of tricomes and adequate ‘polishing’ of the grain by the operator. The entire rubbed sample, including the rubbings, was used to perform the HLM determinations.

The HLM devices used in the study included devices from Germany (Physikalisch – Technische Bundensanstalt, Braunschweig and Berlin, Germany), USA (Seedburo Equipment Co., Chicago, USA), UK (Farm-tec, Whitby, North Yorshire, UK), SA, France

(Chopin Technologies, Villeneuve-la-Garenne Cedex, France), Canada (Dimo’s Tool & Die Ltd., Canada) and Australia (Grain Tec Pty Ltd., Peregian Beach, Queensland, Australia, (Table 3.1). The HLM measurements performed on each device were carried out according to the manufacturers’ instructions. During all measurements care was taken not to tap, knock or shake the respective devices to prevent a falsely high result be obtained. All the HLM measurements were performed by the same operator except for Experiment 6 when the effect of operator was evaluated. In Experiment 6, operator 2 refers to the operator who has conducted all HLM measurements in this study and is referred to as the skilled operator. All HLM measurements were performed on the devices in random order.

Table 3.1 Illustration and a short description of the HLM devices

Country	Description of HLM devices
 **Germany**	Kern 220/222 Grain Sampler with filler and cutter bar (1000 mL measuring cup). Compliant to ISO 7971-2:1995.
 **UK**	Easi-Way Portable Hectolitre Test Weight Kit with cutter bar (500 mL measuring cup). Matched to 20 L volume (Directive 71/347/EC) and conforms to ISO 7971-2:1995 and BS 4371 part 23 standards.
 **France**	Niléma Litre with filling hopper and cutter bar (1000 mL receiving cup). Designed in accordance with the AFNOR NF V 03-719 (1996) standard and standardised to a 50 L French reference.
 **Canada**	Ohaus 500 mL with Cox Funnel and round wooden striker. 500 mL measure with certificate of calibration (calibrations performed traceable to national standard).
 **SA**	South African two-level HLM device with a funnel and 500 mL measuring container (on the lower level) and a round wooden scraper .
 **Australia**	Aluminium 500 mL measure with filler and cutter bar.
 **USA**	Seedburo 151 Filling Hopper with quart cup (1100 mL) and strike-off stick.

Hectolitre mass equipment and operating procedures

The general protocols for use of the respective devices are given below. These protocols were followed when HLM determinations were performed using each of this devices.

German Kern 220/222 Grain Sampler

The sampler is placed on a firm, non-flexible, vibration–free horizontal base. The scraper blade is inserted in the empty 1 L measuring container. Fill the pre-filling measure with the sample of grain up to the level mark. Then empty it to within 3 or 4 cm from the upper edge of the filling hopper in such a way that the grain flows evenly into the middle of the filling hopper in 11 to 13 s. After filling, quickly pull out the straight edge, but without shaking the equipment. When the piston and the grain have fallen into the measuring container, place the straight edge back in the slit and push it through the grain in a single stroke. If a particle becomes jammed between the slit edges, the pouring shall be repeated. Throw out excess grain lying on the straight edge. Then remove the filling hopper and straight edge. Weigh the grain (in grams) and read the HLM in kg.hL^{-1} corresponding, to the weight of the grain, from the conversion chart supplied with the device.

USA Seedburo 151 Filling Hopper with quart cup

The funnel (with valve underneath closed), is filled with enough grain to overflow the measuring container (quart cup = 1100 mL). Open the valve to release the grain into the measuring cup. Move the funnel to the left side of the measuring cup to provide space on top of the cup. Position the wooden striker on the rim of the cup and remove excess grains by means of three swift full-length zigzag motions. Determine the weight of the grain in the measuring container. Convert the weight of the grain in grams to pounds per bushel (lb.bu^{-1}) as indicated on the conversion chart supplied with the device. The obtained value in lb.bu^{-1} is converted to kg.hL^{-1} by multiplying it with 1.287.

South Africa hectolitre mass device

Fill the funnel (valve underneath closed) with enough grain to overflow and scrape off excess grain with the round edge of the wooden scraper. Place the measuring container (500 mL container) on the lower level platform just underneath the funnel. Open the valve to release the grain into the measuring container. Move the funnel to the left to create space above the measuring container. Place the wooden scraper on the rim of the container and scrape off excess grain in one quick, smooth motion. Weigh the mass of the grain in a measuring container. Convert the weight in grams to kg.hL^{-1} by dividing it by 5.

Australian Aluminium 500 mL measure

The receiving container (500 mL) is mounted on top of the measuring container with a whole at its center. Fill both containers with grain through the filler hole. Insert the metal level blade through the slit to isolate the grain from the measuring cylinder underneath. The blade separates a precise volume of grain (below the blade) from excess grain above the blade. The volume of the grain in the measuring cylinder is weighed and converted to kg.hL^{-1} by dividing it by five.

Canadian Ohaus 500 mL measure and Cox funnel

Close the opening of the Cox funnel by inserting the slide into the funnel. Place the funnel on top of the measuring container such that the notched edge of the funnel fits firmly on the rim of the measuring container (500 mL). Fill the funnel with the grain to just more than half way. Remove the slide from the opening of the funnel in one quick motion such that the grain flows into the measuring container. While taking care not to disturb the grain in the measuring container, remove the funnel. Place the round wooden scraper on the rim of the container and scalp off excess grain by means of three full-length zigzag motions. Weigh the mass of the grain in the measuring container in grams and convert to kg.hL^{-1} using the HLM conversion chart supplied with the device.

UK Easi-way Portable Hectolitre Test Weight Kit

Insert a metal cutter into the slit of the container and drop in the piston (plunger weight) such that it rests on the cutter bar in the device. Fill the device with grain at a distance of approximately 2.5 cm above the cylinder. Pull out the cutter bar in one motion such that the piston together with the grain falls to the bottom of the device. Re-insert the cutter bar to separate the grain underneath from excess grain on top of the chamber. Discard excess grain from the cylinder and remove the cutter bar. The weight of the grain in the cylinder in grams is converted to kg.hL-^{1} using the conversion chart supplied with the device.

French Nilèma Litre

Place the hopper on top of the 1 L measuring container such that the notched edge of the hopper is secured on the container. Close the valve underneath the hopper. Fill the hopper with an even flow of the grain. Open and hold the valve to release the grain into the measuring cup. Carefully insert the straight edge cutter bar into the slit. The container must be held firmly to prevent vibration and compaction of the grain. Remove the hopper from the container and weigh the mass of the grain in the container. Divide the mass in grams by 10 to convert to kg.hL^{-1}.

Experiment 1: variation between HLM devices using sub-samples

A schematic layout of the experiment is shown in Fig. 3.1. A 16 kg sample of oats was rubbed as described earlier. The sample was mixed thoroughly by pouring it three times through a Boerner Divider (Seedburo Equipment CO., Chicago, USA). The oats was then divided into 8 times 2 kg sub-samples (of which 7 were used). Each 2 kg sample was tested on each device respectively. The order of devices was chosen at random. Ten HLM measurements were performed on each device. However, between each measurement or repetition, the 2 kg sample was always mixed by pouring it from one bucket to another five times.

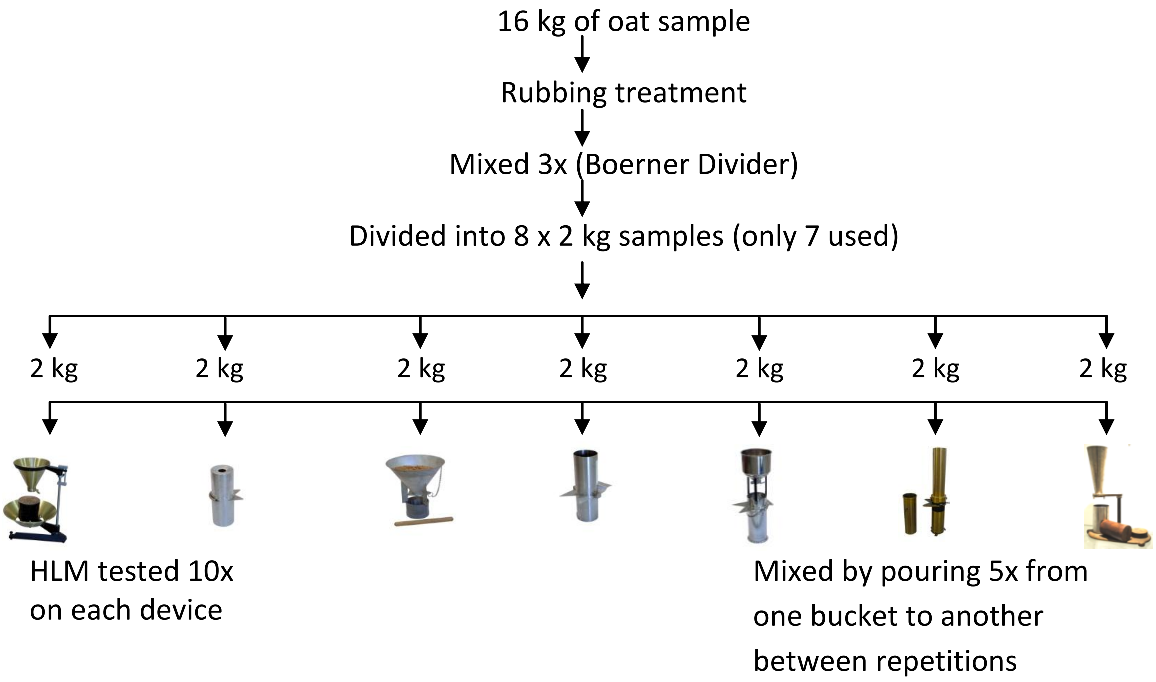


Figure 3.1 Schematic layout of Experiment 1: variation between HLM devices using sub-samples.

Experiment 2: variation in repeatability within and variation between the HLM devices using single work samples

Three different oat samples (16 kg each) with a difference of 3.70 kg.hL^{-1} between the highest and the lowest HLM values were used. After each sample was rubbed, it was mixed thoroughly by pouring it three times through a Boerner Divider. Upon mixing, each sample was divided into 8 times 2 kg samples (of which 7 were used), where each individual sample was tested on each of the HLM devices respectively. The order of the devices was chosen at random. Ten HLM measurements were carried out on each device using individual samples i.e. a separate sample for each device. After the first HLM measurement was done, only the grain needed to do the test was used to execute the remaining nine repetitions. The two remaining samples were analysed similarly. A schematic layout of this experiment is shown in Fig. 3.2.

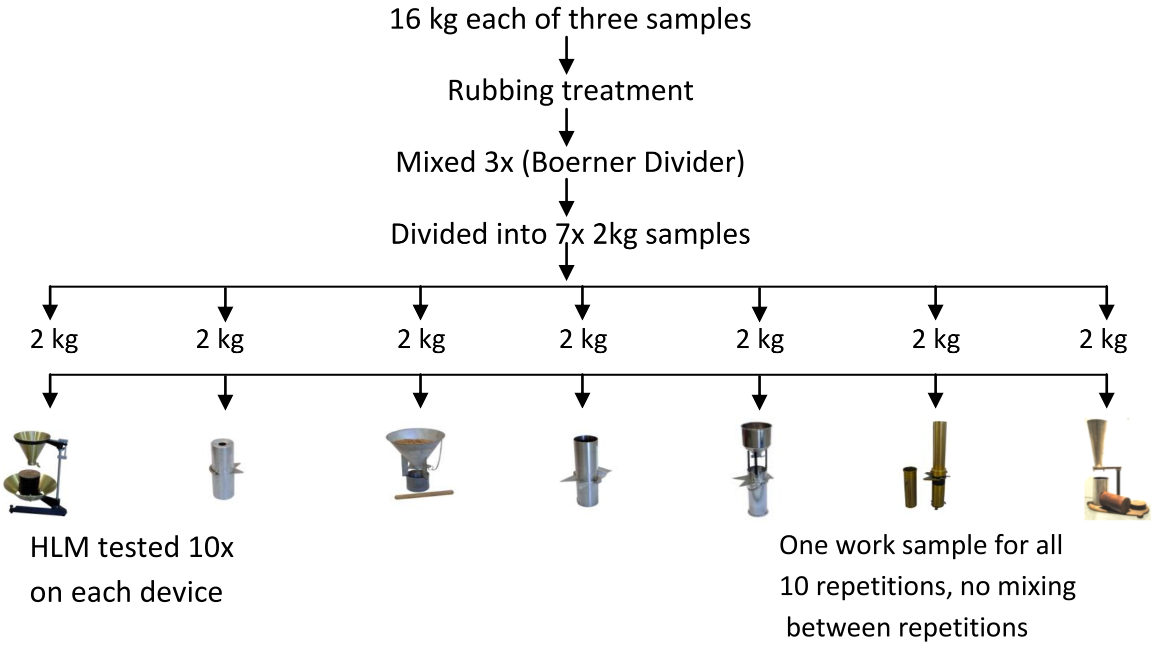


Figure 3.2 Schematic layout of Experiment 2: variation in repeatability within and variation between the HLM devices using single work samples.

Experiment 3: comparison of the HLM devices using a single work sample of 10 oat samples

In this experiment (Fig. 3.3), ten different oat samples (6 kg each), with a range of 10.50 kg.hL^{-1} between the highest and the lowest HLM values were used. Each of the samples was rubbed and mixed three times using a Boerner Divider. After mixing, each of the 6 kg sample was divided into three times 2 kg sub-samples to be tested on each device respectively. A work sample of the same 2 kg sub-sample was tested repeatedly on all the devices. The first HLM measurement was always performed on the USA device because it requires more grain to do the test than the other devices. This work sample was subsequently used to do HLM tests on the other devices. After the first test was done on the USA device, the order of testing on the other devices was chosen at random. Each sub-sample was measured in duplicate on each respective device, resulting in 6 HLM values per sample per device.

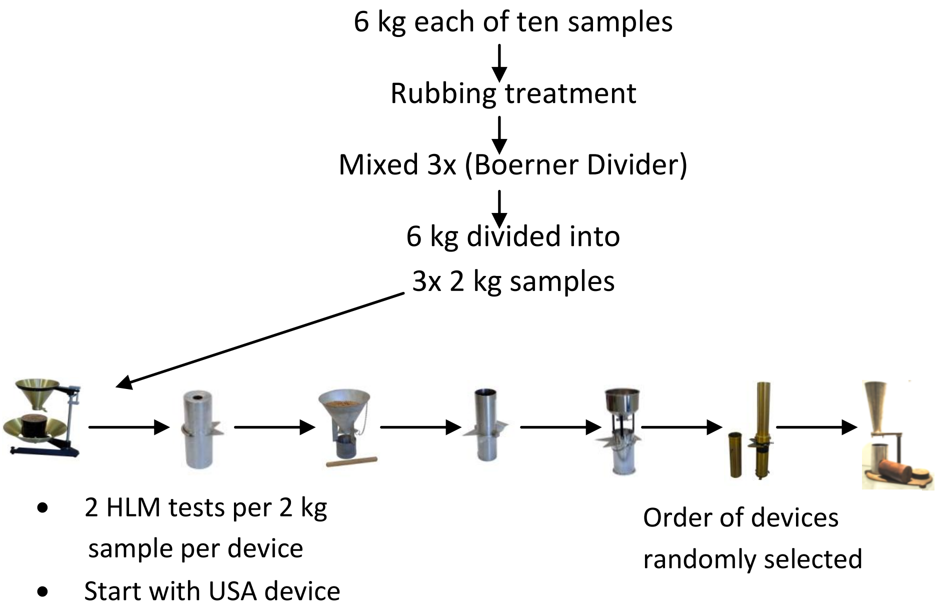


Figure 3.3 Schematic layout of Experiment 3: comparison of the HLM devices using a single work sample of 10 oat samples.

Experiment 4: comparison between the German and South African HLM devices using sub-samples

Each of the three different oat samples (8 kg each) with a difference of 5.93 kg.hL^{-1} between the highest and the lowest HLM values, were mixed by pouring it three times through the Boerner Divider. This was done after the samples were rubbed. After mixing, each 8 kg sample was divided into four times 2 kg sub-samples to be tested individually on each device. Ten HLM measurements were executed on each device of two German and two South African devices. However, in between measurements, the 2 kg sample was always mixed by pouring it from one bucket to another five times. The schematic layout of this experiment is shown in Fig. 3.4.

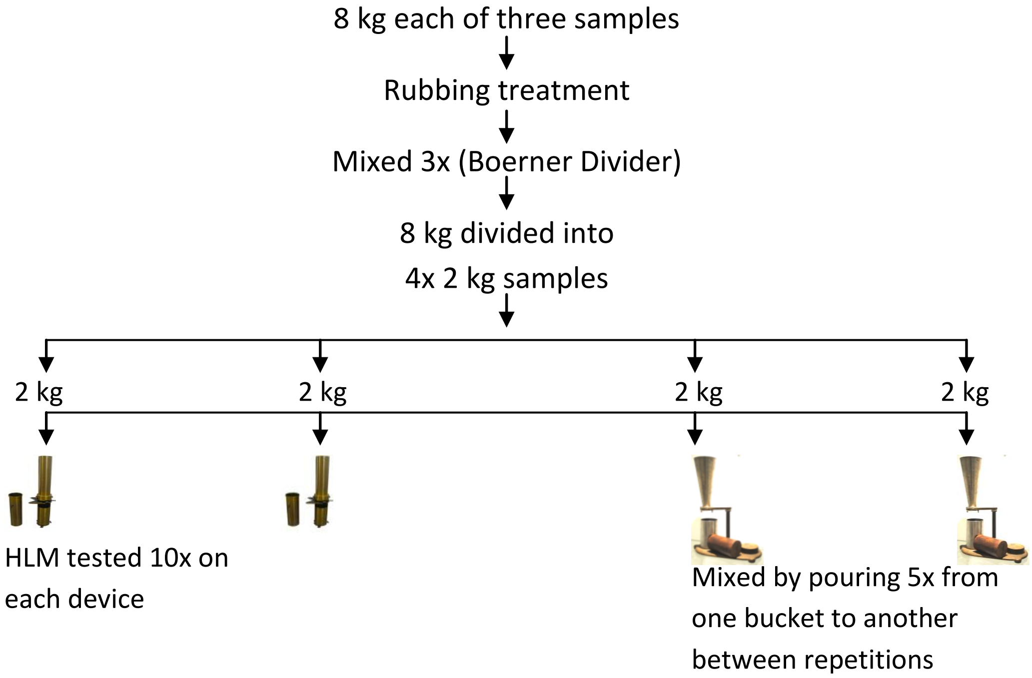


Figure 3.4 Schematice of Experiment 4: comparison between German and South African HLM devices using sub-samples.

Experiment 5: effect of rubbing of oat samples on HLM measurements using sub-samples

Fig. 3.5 shows a schematic layout of experiment 5. Three different samples (16 kg each), with a range of 5.85 kg.hL^{-1} between the highest and the lowest HLM values, were used in this experiment. Each of the samples was poured through a Boerner Divider three times in order to get well-mixed eight times 2 kg sub-samples (7 samples were used). With no rubbing done on the samples, each individual 2 kg sub-sample was analysed on each device. The order of the devices was chosen randomly. Ten HLM measurements were performed on each device for each respective sample. However, between repetitions, the 2 kg sample was mixed by pouring it from one bucket to another five times. After the HLM measurements were done, each 2 kg sample was rubbed as described earlier. All the rubbed 2 kg samples were analysed in a similar way to the unrubbed samples.

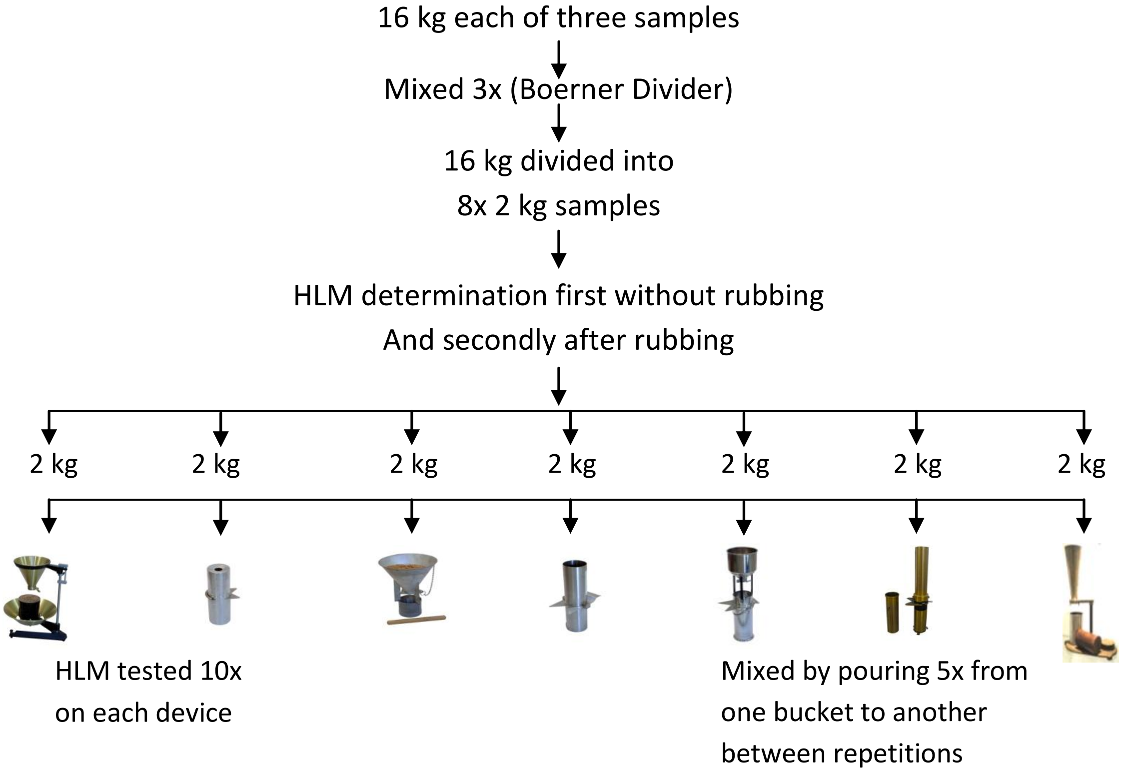
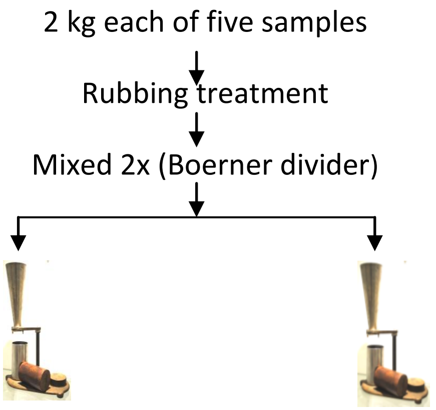


Figure 3.5 Schematic layout of Experiment 5: effect of rubbing of oat samples on HLM measurements using sub-samples.

Experiment 6: effect of operator on HLM determinations

Five oat samples (8 kg each) with a range of 8.88 kg.hL^{-1} between the highest and the lowest HLM values were used in this experiment (Fig. 3.6). Each of the samples was poured through a Boerner Divider two times in order to get well-mixed samples. This was done after the samples were rubbed. Three different operators, with three levels of competency (skilled, semi-skilled and skilled), performed HLM measurements using the same samples. HLM measurements were conducted on two SA HLM devices. The work sample obtained from the first measurement with each sample done by the first operator was used for testing the other device and was kept for the other operators to conduct their HLM measurements. Each operator performed ten repetitions, but for each repetition the samples were randomly selected.



- 3 operators (skilled, semi-skilled, & unskilled) each performed 10 tests on each device, using 5 different samples.
- Order of samples randomly selected
- A single work sample used for all

Figure 3.6 Schematic layout of Experiment 6: effect of operator on HLM determinations

Experiment 7: relationship between protein content and HLM values of oats

The protein content of ten oat samples, with a difference of 11.05 kg.hL^{-1} in HLM, was determined. The oat samples (15 g) was milled for one minute using a laboratory mill (Retsch model ZMI, Haan Germany) fitted with a 0.5 mm mesh size ring sieve. The ground sample was transferred into a container, mixed with the spatula and covered with parafilm until being weighed. The Dumas combustion analyser was used (Model Truspec® N elemental Determinator, Leco Africa, Kempton Park, South Africa). Ethylenediaminetetraacetic acid (EDTA) with a known nitrogen content of 9.57% was analysed prior to protein determination. The EDTA standard (0.10 ± 0.001 g) was weighed into a tin foil sample cup, twisted and rolled into an egg shape and placed on the carousel loading head of the instrument. The EDTA was used to calibrate the instrument. The same procedure was followed for the whole oat meal except that 0.35 ± 0.001 g was weighed into a tin foil cup. A 6.25 conversion factor was used to convert from nitrogen to protein content. The protein content was then expressed on a 12% moisture basis (mb). Protein analysis were performed in duplicate.

Moisture content determination

Moisture contents (determination of the weight loss of a sample when dried at 130°C under specified conditions) were performed according to an adapted method of the AACC 45-15A method (AACC, 2004). Moisture dishes were dried in a vacuum oven (Heraeus Model RVT 360, Henau, Germany) at 130°C for 30 min and cooled in a desiccator for 40 min. The weight of the pre-dried moisture dishes with lids were determined (recorded to at least 0.001 g). The sample to be dried (5 ± 0.001 g) was transferred into the moisture dishes. Moisture dishes were placed in the vacuum oven with lids next to them and samples were dried at 130°C for one hr. Afterwards, the moisture dishes were removed from the oven, covered with lids and transferred to the desiccator to cool for 45 min. The new mass of the covered moisture dishes with dried sample was recorded to the nearest 0.001 g. Moisture content was calculated as the loss in weight, expressed as a percentage of the weight of the original sample using the following formula.

$\% \text{ moisture} = [W_2 - W_3 / W_2 - W_1] \text{ X } 100$

where

W_1 = mass of moisture dish; W_2 = mass of moisture dish + sample before drying; and
W_3 = mass of moisture dish + sample after drying.

For each sample, the moisture content was done in duplicate.

Experiment 8: effect of consecutive wetting and drying of oat samples on HLM results

Four samples (8 kg each) with a range of 10.91 kg.hL^{-1} between the highest and the lowest HLM values were rubbed. Each 8 kg sample was divided into four sub-samples of 2 kg each. Three of the four sub-samples were conditioned to a moisture content of *ca.* 14, 16 and 18% respectively. The remaining sample was kept at its original moisture content (*ca.* 10%) (Fig. 3.7). The samples were conditioned by adding appropriate amount of deionised water to obtain the desired moisture contents, respectively. The starting point of wetting the grain was to determine its original moisture content. This was used to determine the amount of water needed to wet the grain to the respective moisture content levels. The amount of water required to wet the grain was calculated using the following formula:

$$\text{Required } H_2O \text{ (mL)} = \frac{\text{mass (g)} \times [\text{target moisture \% - initial moisture \%}]}{[100 - \text{target moisture content}]}$$

where

Required H_2O (mL) = amount of water to be added to oats to reach a desired moisture content.
Mass (g) = mass of oat to be conditioned;
Target moisture % = *ca.* desired moisture content of oats after conditioning; and
Initial moisture % = original moisture content of oats before conditioning.

Researchers know from experience that when the calculated amount of water is added to the oats, it does not result in the expected moisture content. It is presumably due to the increase in total mass of the wetted oats after the water has been added to the oat samples. It was suggested that an additional 8% of the calculated amount of water was added to correct for this difference. Immediately upon adding the water, the samples were mixed thoroughly by shaking the containers for five min. The containers were constantly shaken at regular intervals for the first one hr of wetting. The samples were left to equilibrate at ambient temperature for 24 hrs. After 24 hr, the samples were mixed again for five min and stored at 4°C for an additional 24 hr. The samples were then allowed to equilibrate to ambient temperature and relative humidity. This was achieved by spreading the samples in a single layer for one hr. The moisture contents of the conditioned oat samples were confirmed according to the one-hour oven method.

The HLM of all sub-samples were measured in duplicate on each of the seven different devices. The first measurement was performed on the USA device after which the work sample obtained was used to perform HLM measurements on the other devices in random order. All the samples were then dried in a forced circulation heating room at *ca.* 35°C to a moisture content *ca.* 10%. The moisture contents of the dried samples were again confirmed by the one-hour oven method and the HLM of all the samples again determined as described above. All the samples were again conditioned to their original moisture content i.e. original, 14, 16 and 18%, respectively, before drying. The moisture contents of all samples after each respective moisture treatment are shown in Table 3.2.

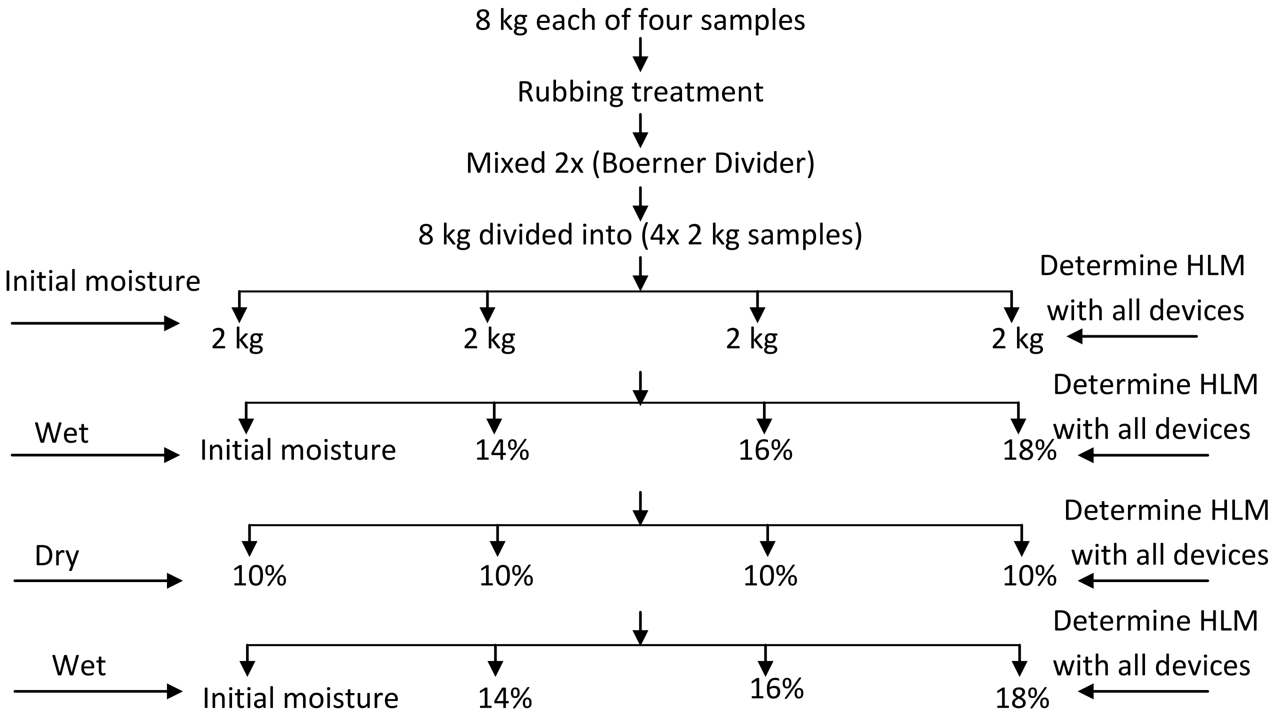


Figure 3.7 Schematic layout of Experiment 8: effect of consecutive wetting and drying of oat samples on HLM results.

Table 3.2 Average moisture contents of oat samples after respective moisture treatments

Moisture treatments	**Moisture content (%)**			
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Initial moisture	11.5	10.8	11.3	10.9
Conditioned to *ca*. 14%	13.5	13.7	13.3	13.9
Conditioned to *ca*. 16%	15.3	15.8	16.1	15.1
Conditioned to *ca*. 18%	17.6	17.0	16.9	17.5
Drying initial to *ca*. 10%	9.7	9.4	10.1	10.2
Drying 14% to *ca*. 10%	9.2	9.8	9.8	9.7
Drying 16% to ca. 10%	10.1	9.9	10.2	10.2
Drying 18% to *ca*. 10%	10.9	9.8	10.6	9.9
Conditioned back to initial moisture	11.2	10.4	11.5	10.5
Conditioned back to *ca*. 14%	14.2	13.8	13.7	14.4
Conditioned back to *ca*. 16%	15.6	15.3	15.8	15.2
Conditioned back to *ca*. 18%	17.0	17.4	17.2	17.6

Experiment 9: effect of consecutive wetting and drying cycles on the microstructure of oats using scanning electron microscopy

The effect of consecutive wetting and drying cycles on the microstructure of oats was investigated using a scanning electron microscope (SEM). One oat sample (2.8 kg) with a HLM value of 50.05 kg.hL^{-1} was obtained. This sample was divided into four times 700 g sub-samples after being rubbed. The four sub-samples were conditioned and dried as discussed in Experiment 8. The moisture content was determined using the one-hour oven method as described earlier. The HLM measurements were done on the German device. Micrographs of the samples were accomplished using a Leo® 1430VP Scanning Electron Microscope (Zeiss, Germany). A single kernel was selected randomly for each of the moisture treatments, respectively. Prior to taking the micrographs, the samples were sputter-coated with gold. Samples were identified with secondary electron images. Beam conditions during imaging were 7 KV and approximately 1.5 nA, with a working distance of 13 mm and a spot size of 150. Representative micrographs were taken at the center and closer to the edge of the endosperm of the same kernel at a magnification of 1000x and 3000x, respectively.

Statistical analysis

Statistical analysis were performed and graphs compiled using Statistica version 10.0 (StatSoft, Inc., Tulsa, OK, 74104, USA). Repeated measure of analysis of variance (RANOVA) was performed to compare average measurements between instruments to determine absolute difference. The vertical bar represents the 95% confidence interval for the average measurements. Fisher least significant difference (LSD) post-hoc testing was used. All references to significant differences indicate statistical differences. Additionally the intra-class correlation (ICC) coefficients were determined as the ICC agreement that correlates measurements, while taking into account the differences in absolute values of the respective measurements, and the ICC consistency that only correlates measurements. All ICC calculations were done using R statistical language.

Results

Experiment 1: variation between HLM devices using sub-samples

Fig. 3.8 shows the mean HLM values obtained from the respective devices when only one oat sample was used. The average HLM measurements obtained from the German, Canadian, Australian, UK and French devices did not differ significantly ($P>0.05$). However, significantly ($P<0.05$) lower values were obtained from the SA and USA devices. The latter two devices also differed significantly ($P<0.05$) from each other.

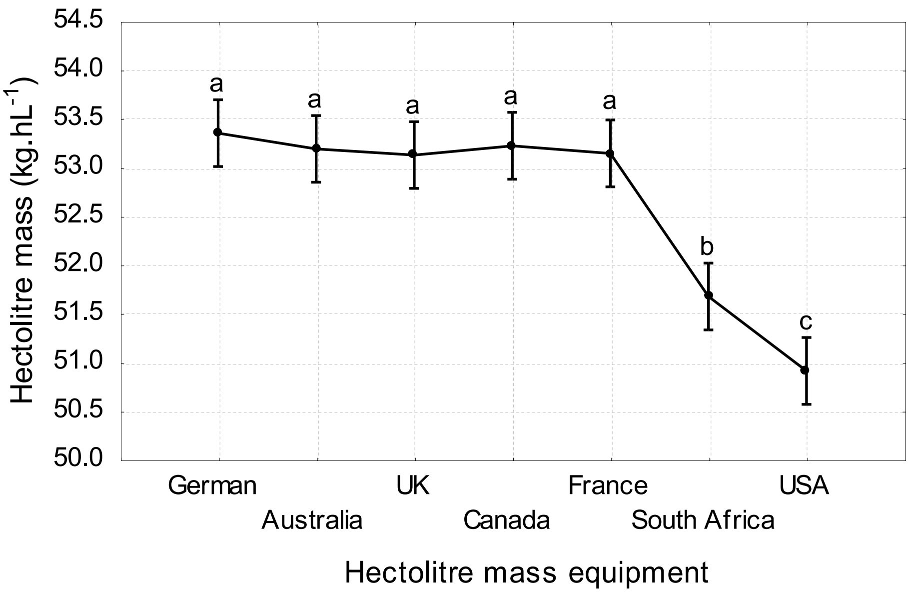


Figure 3.8 Differences between the average hectolitre mass (HLM) values obtained with the HLM devices using a single oat sample, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

Experiment 2: variation in repeatability within and variation between the HLM devices using single work samples

Irrespective of the device and/or sample used, no significant differences (*P*>0.05) were observed between the ten repetitions performed on any of the samples with any of the devices. Examples of results obtained can be seen in Fig. 3.9. This indicates irrespective of the number of times a sample went through a device, the HLM values did not change significantly between the first and the last analysis. This confirmed efficient sample preparation in terms of rubbing, mixing and sampling. All samples used in the remaining experiments were thus prepared in the same way.

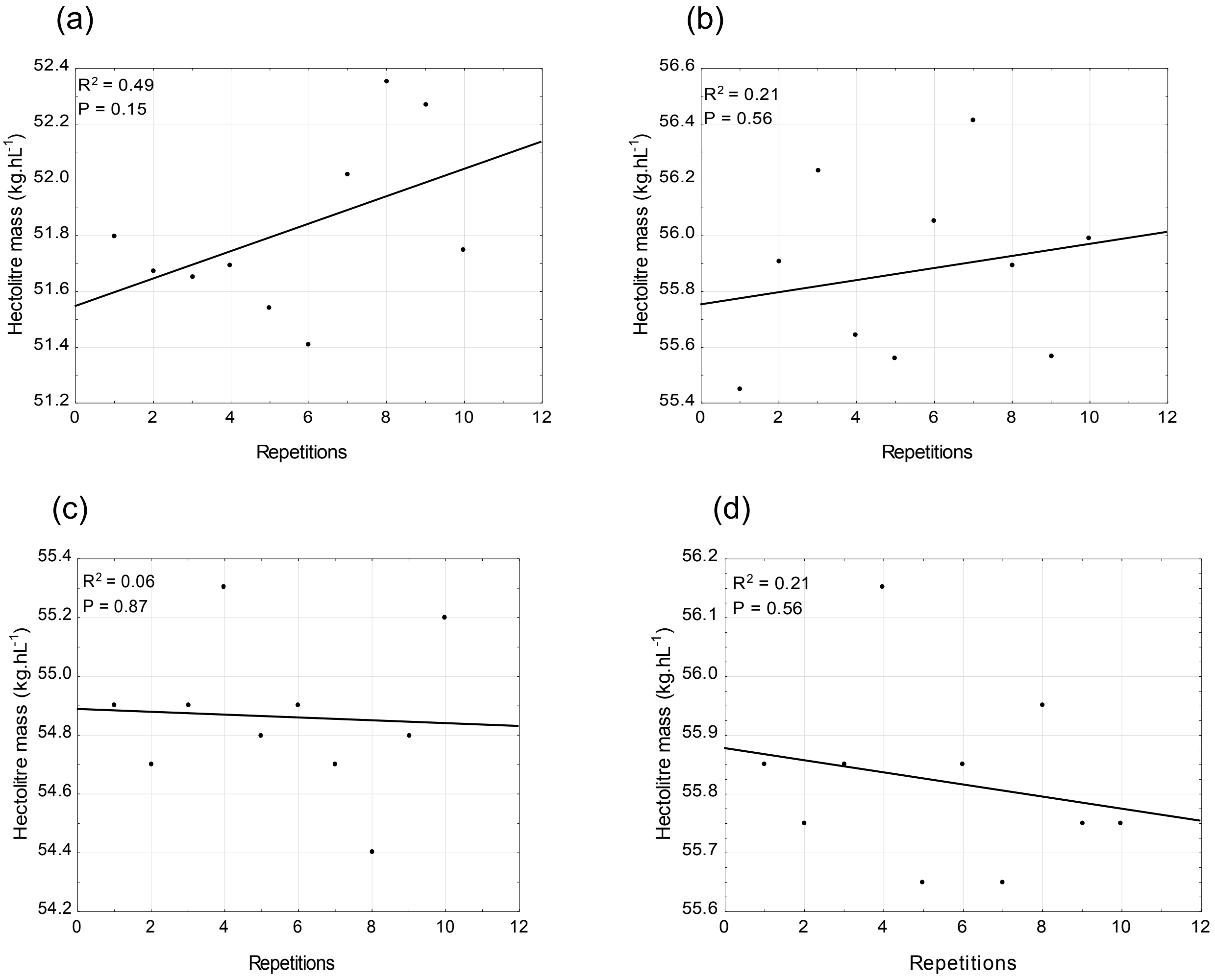


Figure 3.9 Regression scatter plots showing the differences in hectolitre mass (HLM) values of the ten successive measurements obtained with (a) the South African device using sample 1; (b) the South African device using sample 2; (c) the German device using sample 1; and (d) the German device using sample 3.

Average HLM measurements for the three respective oat samples as determined for each device are shown in Fig. 3.10. Again the SA and USA devices resulted in significant lower ($P<0.05$) HLM values; this was irrespective of the sample used. Fig. 3.11 shows the differences between the average HLM values obtained from the three oat samples. Significant differences ($P<0.05$) were observed between most of the devices. This was mostly due to a single work sample being used resulting in very little variation within each sample. These small differences also indicate high repeatability within each of these devices. Thus if the same sample is analysed repeatedly the same result is obtained. Distinctly lower average HLM values were obtained with the SA and USA devices.

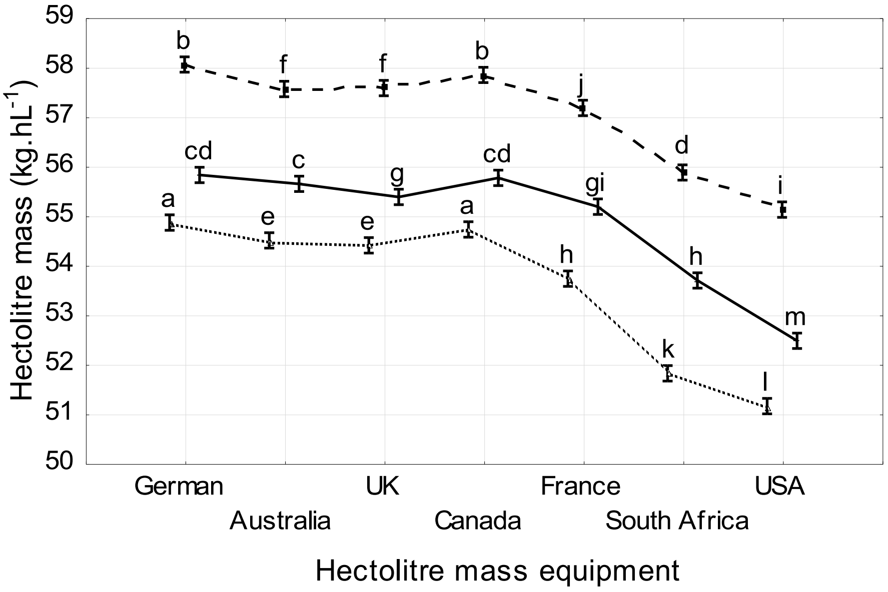


Figure 3.10 Differences between the average hectolitre mass (HLM) values obtained from the three oat samples, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

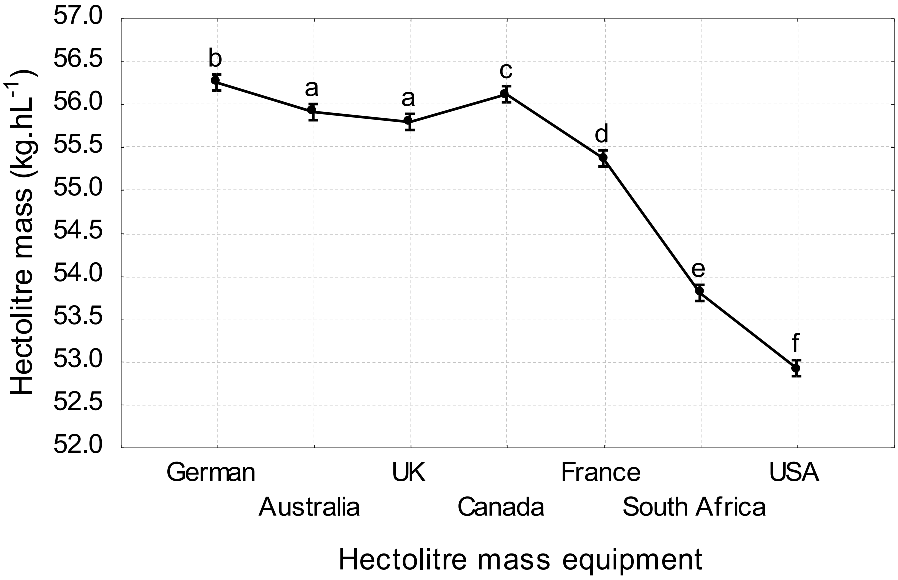


Figure 3.11 Differences between the average hectolitre mass (HLM) values obtained from the three oat samples, determined with repeated analysis of variance (RANOVA). Different letters indicates significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

The average ICC agreement and ICC consistency values are shown in Table 3.3. Similar average ICC agreement results were obtained for the German, UK, Canadian, French and Australian devices. The low values of approximately 0.7 would have been due to the significant differences obtained due to single work samples being used. The SA and USA devices had much lower average ICC agreement values (0.56 and 0.43, respectively) indicating a substantial difference in actual values compared to the other devices. In spite of the devices producing different HLM values, they were highly correlated with ICC consistency values of more than/and or equal to 0.94. The differences in the values observed between the devices can be attributed to the different operating procedures of the devices and the different volumes of the receiving cups or receptacles. The German device is equipped with a 1 L measuring cup where as the SA device has a 500 mL measuring cup. The error of packing grain into the larger measuring cup of the German device compared to the 500 mL cup of the SA device would have contributed to differencess in mean HLM values of these two devices. It is also believed that variation between the instruments and operator errors in measurement can arise from the manner in which the grain is poured into the measuring container and the manner in which the grain packs into the measuring container. During the measurements, any vibrations, shaking or knocking of the instrument was avoided even though the latter could not be hundred percent controlled.

Table 3.3: Intra-class correlation (ICC) agreement and ICC consistency for the respective hectolitre mass (HLM devices) for the variation in repeatability within and variation between the HLM devices using single work samples

HLM device	Average ICC agreement	Average ICC consistency
German	0.72	0.96
Australia	0.75	0.94
UK	0.76	0.95
Canada	0.73	0.95
France	0.76	0.97
SA	0.56	0.94
USA	0.43	0.96

Experiment 3: Comparison of the HLM devices using a single work sample of 10 oat samples

No significant differences (*P*>0.05) in HLM values were observed between the three sub-samples obtained (Fig. 3.12). This indicates that the rubbing and obtaining the sub-samples, using the Boerner Divider was done efficiently and that the three sub-samples were representative of the respective bulk oat samples. From this it is clear that the longer rubbing period of three min did not influence the results. Fig. 3.13 shows the differences between the average measurements for the HLM devices. As before, the SA and USA devices resulted in significantly lower (*P*<0.05) HLM values. Although the UK and French devices also differed significantly, it would not have any practical impact. The reason again, due to single work samples (almost no variation within the sample) being used, even very small differences were shown as being significant.

The differences in actual average HLM values as obtained with the respective devices have also been evaluated by means of the ICC agreement and ICC consistency (Table 3.4). An average ICC agreement of 0.88 and ICC consistency of 0.99 were obtained. Thus, although actual differences between the devices have been observed, the measurements obtained with the devices were highly correlated. Individual results between the respective devices are shown in the Appendix 3, Table 4. It is clearly shown that the South African device resulted in HLM values distinctly different to the other devices (apart from the USA device).

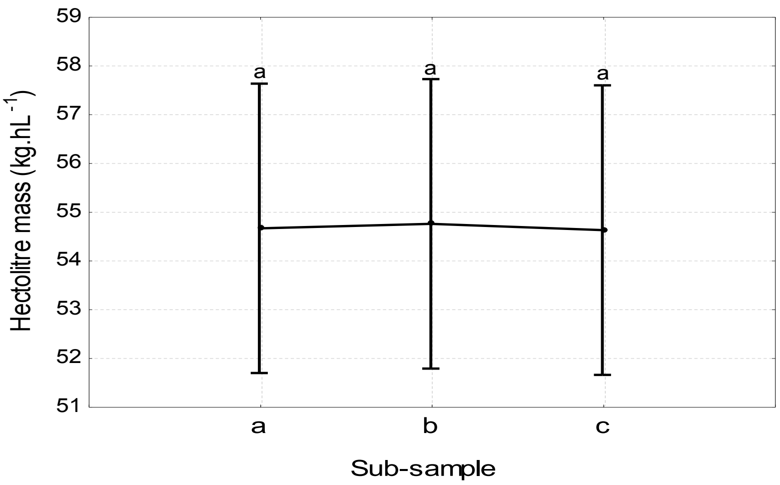


Figure 3.12 Differences between the average hectolitre mass (HLM) values, obtained for the three sub-samples, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

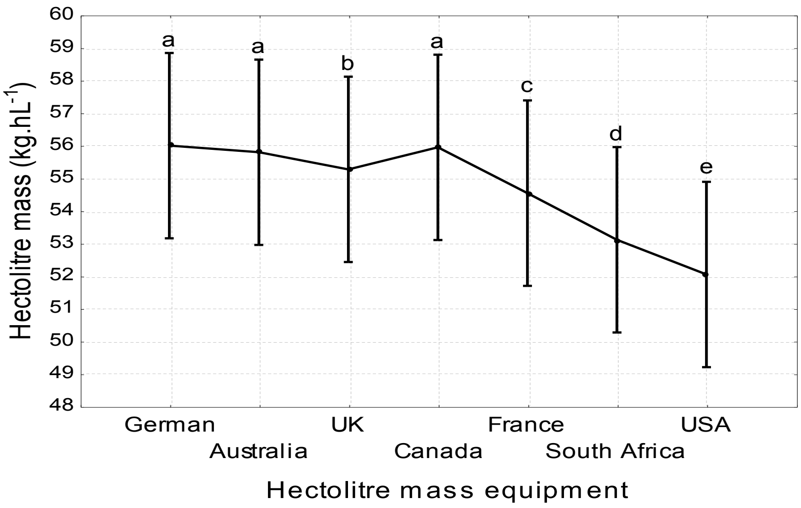


Figure 3.13 Differences between the average hectolitre mass (HLM) values obtained with the HLM devices using single work oat samples, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

Experiment 4: Comparison between two German and two South African HLM devices using oat sub-samples

As expected, the two German devices resulted in similar HLM values ($P>0.05$). Similarly, the two SA devices did not differ significantly ($P>0.05$) (Fig. 3.14). The average HLM values of the Germany devices were higher (3.12 kg.hL^{-1}) than those of the SA devices (Fig. 3.15). The average HLM values of ten repetitions for all four devices are shown in Fig. 3.16. This shows good repeatability within instruments with only one measurement being significantly ($P<0.05$) lower.

Table 3.4 Intra-class correlation (ICC) agreement and ICC consistency between the hectolitre mass (HLM) values as determined with the HLM devices using 10 single work oat samples

HLM equipment		**ICC agreement**	**ICC consistency**
Germany	Australia	0.99	0.99
Germany	UK	0.98	1.00
Germany	Canada	0.99	0.99
Germany	France	0.94	0.99
Germany	SA	0.81	0.99
Germany	USA	0.70	0.99
Australia	UK	0.98	0.99
Australia	Canada	0.97	0.97
Australia	France	0.94	0.97
Australia	SA	0.82	0.97
Australia	USA	0.71	0.98
UK	Canada	0.98	0.99
UK	France	0.97	0.98
UK	SA	0.88	0.99
UK	USA	0.78	0.99
Canada	France	0.94	0.99
Canada	SA	0.81	0.99
Canada	USA	0.70	1.00
France	SA	0.94	1.00
France	USA	0.85	1.00
SA	USA	0.97	1.00
Overall		0.88	0.99

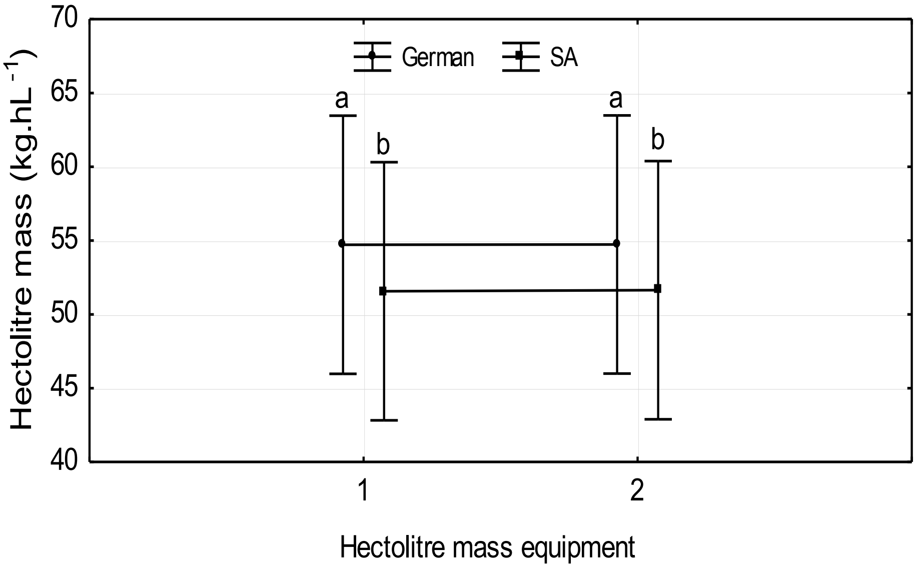


Figure 3.14 Differences between the average hectolitre mass (HLM) values obtained with two German devices (German 1 & German 2) and two South African devices (SA 1 & SA 2), determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

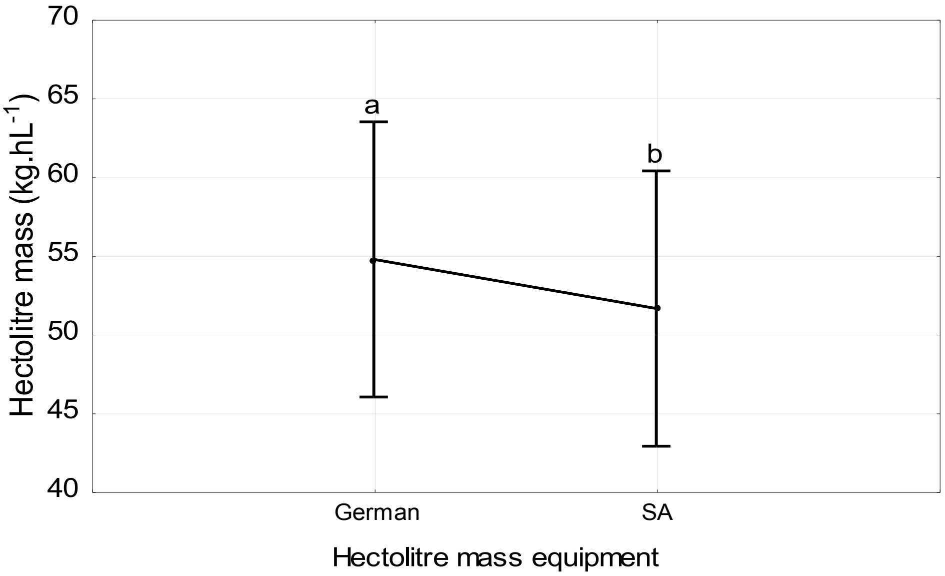


Figure 3.15 Differences in average hectolitre mass (HLM) values between the German and the South African HLM devices, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals

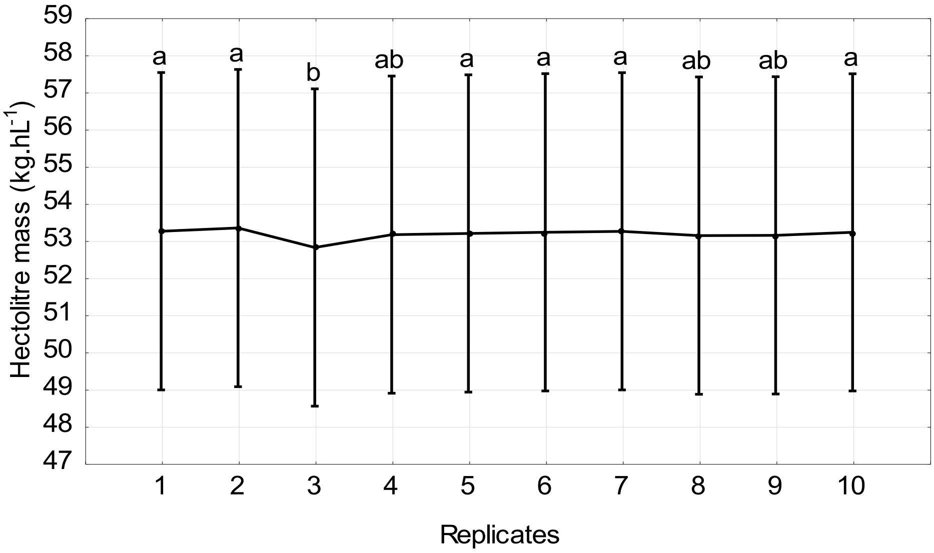


Figure 3.16 Differences between the average hectolitre mass (HLM) values of ten repetitions combined obtained for two German and two South African HLM devices, determined by repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals

Experiment 5: effect of rubbing of oat samples on HLM measurements using sub-samples

According to the draft regulations of oats in South Africa, rubbing should be done for one min. For the purpose of this study rubbing was done for three minutes. Since all the samples were treated similarly, and results were compared with each other, they could still be interpreted effectively. From the RANOVA results (Fig. 3.17 & 3.18) it was clear that the average HLM values significantly increased ($P<0.05$) when samples were rubbed before HLM measurements were performed. The German and Canadian devices gave average HLM measurements that were not statistically different ($P>0.05$) from one another, even though significantly higher ($P<$ 0.05) than the rest of the other devices. Similar to results of earlier experiments, both the SA and USA devices produced significantly lower ($P<$ 0.05) average HLM values compared to the other devices.

The significant increase in average HLM values when samples were rubbed were due to changes in shape and size that the oat grain undergoes upon rubbing. The HLM of oats could be significantly increased by mechanically rubbing, clipping off the tips of oat grain and polishing the oat grain. The reason being that oats with tippy hulls or awns have more air space between them. Thus clipping off the tips; rubbing or polishing would decrease empty spaces between the oat grain and act to improve packing efficiency and HLM of oats.

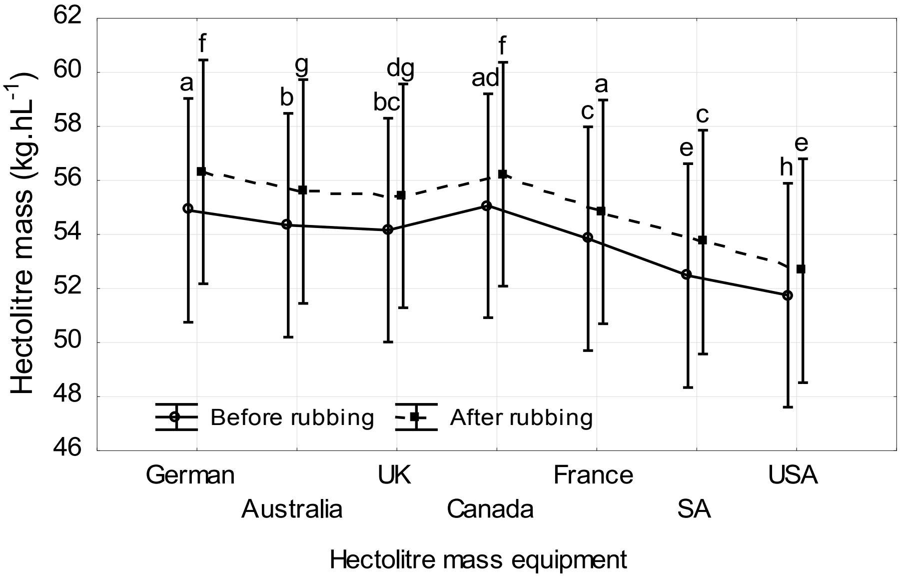


Figure 3.17 Differences between the average hectolitre mass (HLM) values of the three oat samples before and after rubbing, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

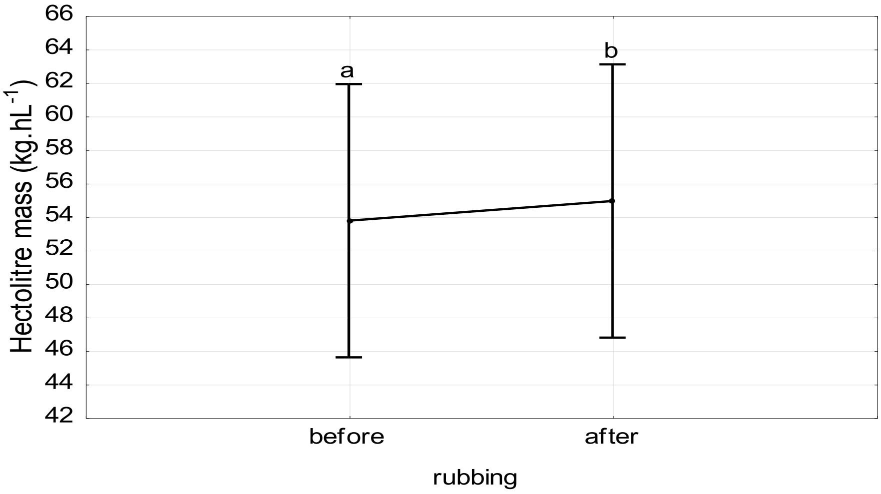


Figure 3.18 Differences between the average hectolitre mass (HLM) values of the oat samples combined before and after rubbing, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals

Experiment 6: effect of operator on HLM determination

The results showed that there was no significant (*P*>0.05) operator effect between the skilled (operator 2) and semi-skilled operator (operator 1) (Fig. 3.19). The unskilled operator (operator 3) measured significantly higher average HLM values on both devices compared to the other operators. ICC agreement value of 0.98 and ICC consistency value of 0.99 were observed for the unskilled operator on the first SA device. These values were found to increase to 0.99 (ICC agreement) and 1.00 (ICC consistency) for the results obtained on the second SA device by the same unskilled operator.

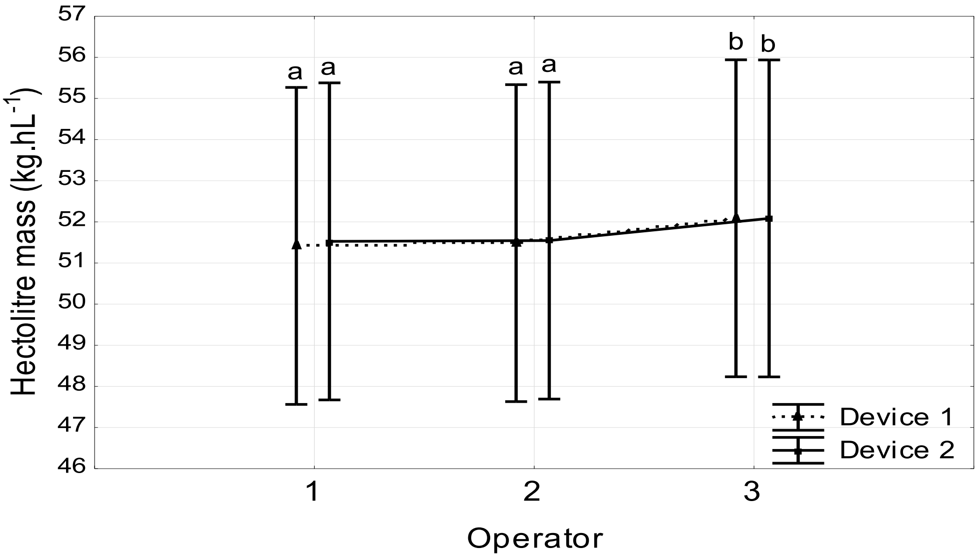


Figure 3.19 Differences between the combined average hectolitre mass (HLM) values obtained by three operators determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

Experiment 7: relationship between protein content and HLM of oats

The results showed that the protein content ranged from 6.80 to 10.56% (Fig. 3.20). Poor correlation (r = 0.18) existed between the HLM and protein content of oats. For example the sample with the lowest HLM value (48.65 kg.hL^{-1}) had a higher protein content than the highest HLM sample (60.20 kg.hL^{-1}).

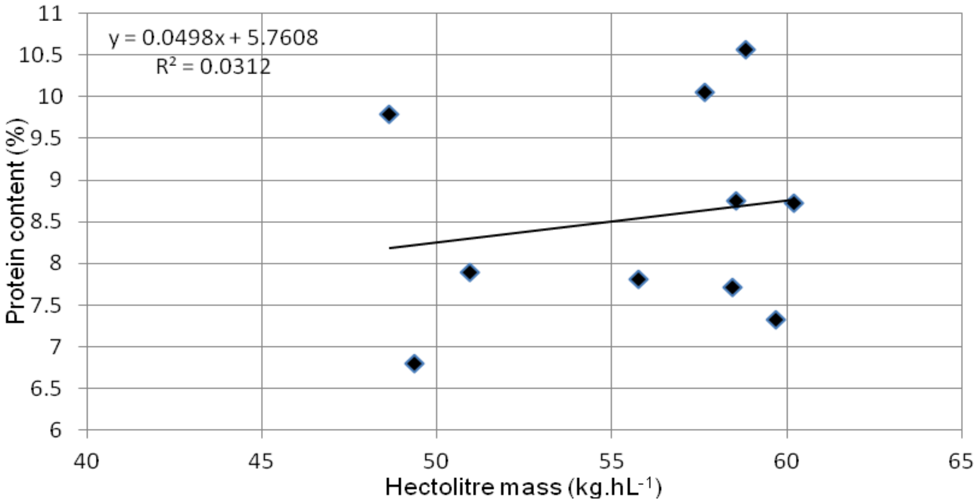


Figure 3.20 Relationship between hectolitre mass (HLM) and crude protein content for 10 oat samples.

Experiment 8: effect of consecutive wetting and drying of oat samples on HLM results

The HLM values decreased significantly ($P<0.05$) from 53.79 to 47.62 kg.hL^{-1} as the moisture content increased from *ca*. 10 to 18% (Fig. 3.21). The HLM values of the control samples (which did not receive any treatment before drying) increased slightly, although not significant after being dried to *ca*. 10% (Fig. 3.22). When these samples were conditioned back to their original moisture contents after drying, their HLM value decreased slightly below the HLM value obtained at its initial moisture content. This would have been expected since these changes in moisture content were really small. Similarly, HLM values of samples of which the moisture contents were increased to *ca.* 14% did not change significantly after been dried and conditioned again. In contrast, the samples which were conditioned to *ca*. 16 and 18% moisture contents, dried to *ca*. 10% and conditioned *ca*. 16 and 18% did result in significantly lower ($P<0.05$) HLM values after the second conditioning. Thus, the greater the change in moisture, the more severe the resulted effect on the HLM measurement of oats. This can be clearly seen in Fig 3.22.

The decrease in HLM values observed after the second wetting could be due to the swelling and roughening of the hulls of the oat grain. Also during hydration the grain expands resulting in less kernels required to fill the test container. The expanded kernels, however, do not contract to their original size on drying. Their bran layer and hulls get loosened and the exterior structure of the kernels is disturbed.

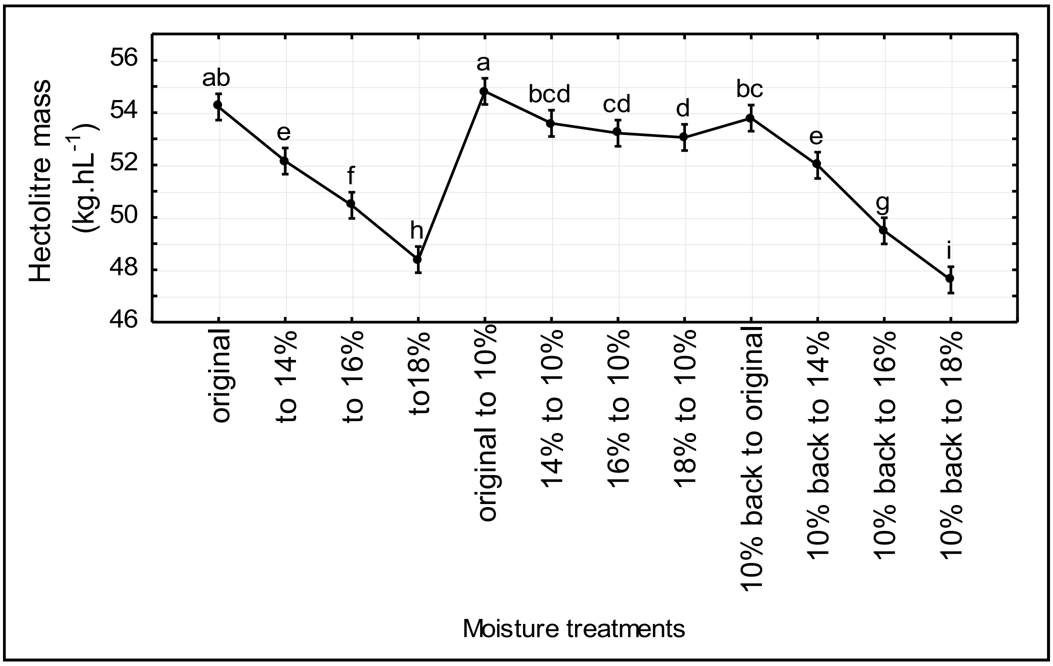


Figure 3.21 Differences in the average hectolitre mass (HLM) values after consecutive wetting and drying cycles of oat, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

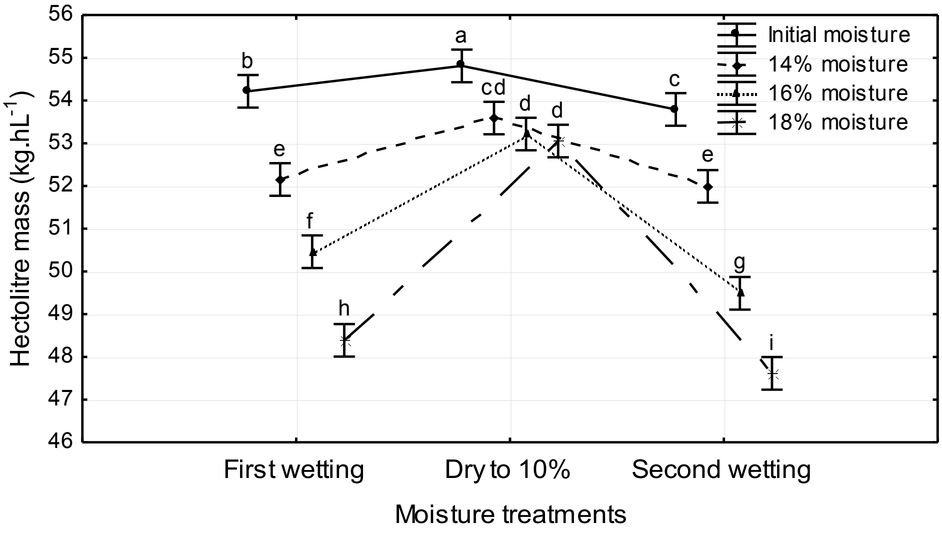


Figure 3.22 Differences in the average hectolitre mass (HLM) values after wetting (1st & 2nd wetting) and drying cycles, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

Differences in average HLM values, obtained by the respective devices, when samples where analysed at different moisture contents are shown in Fig. 3.23 and Fig. 3.24. As was observed in earlier experiments the SA and USA devices resulted in lower HLM values.

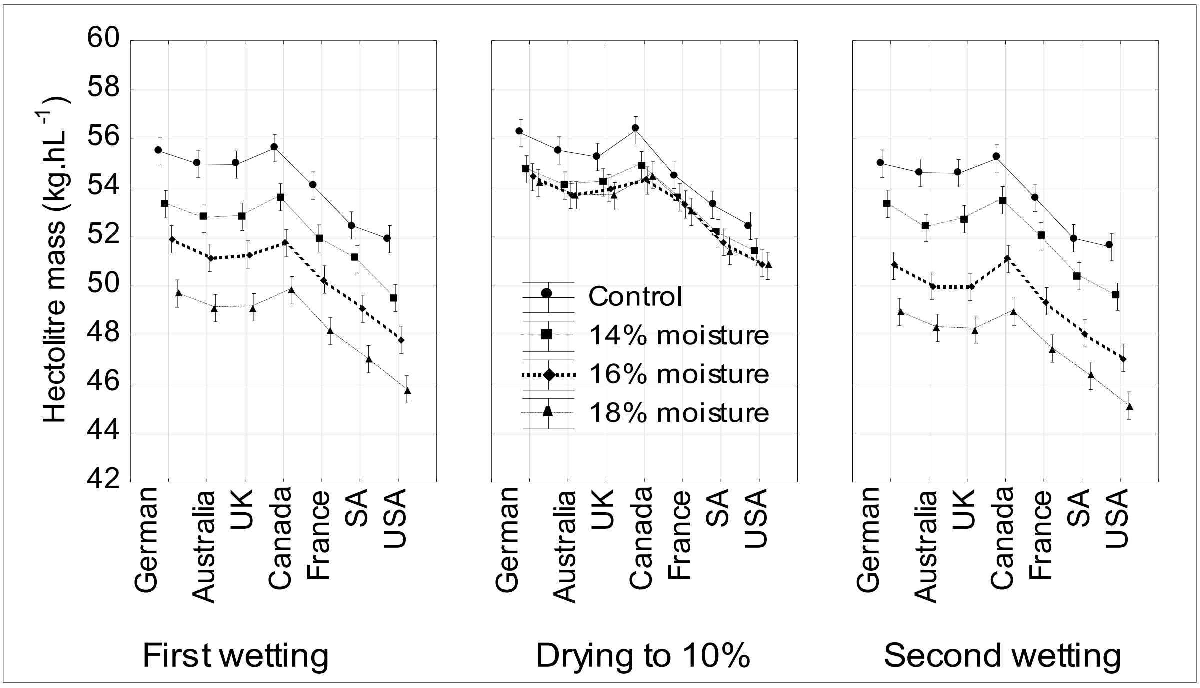


Figure 3.23 Differences in the average hectolitre mass (HLM) values of samples that have undergone wetting and drying cycles, determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

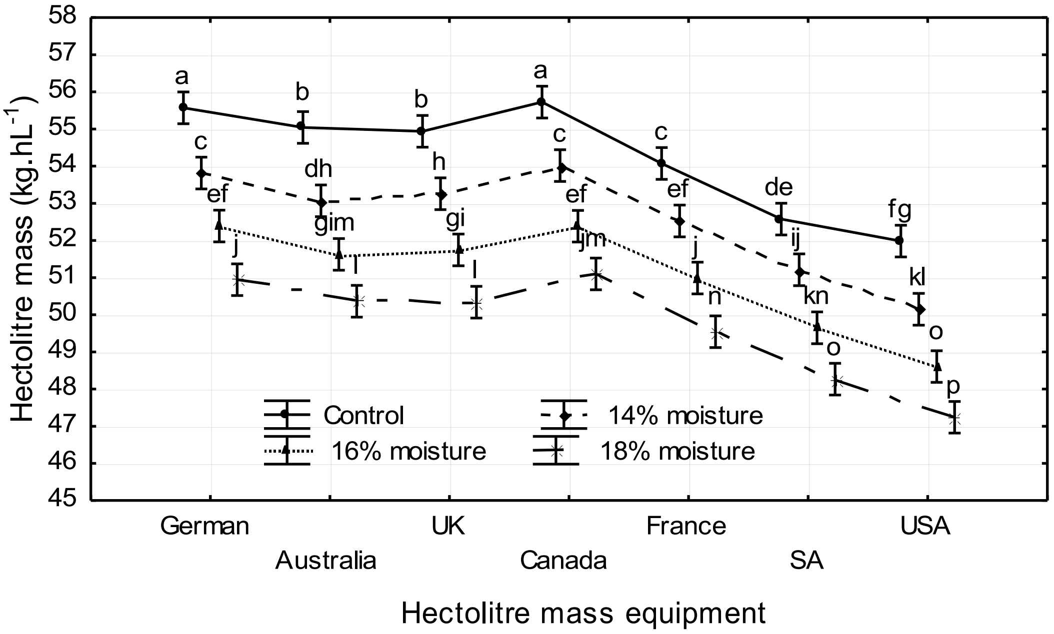
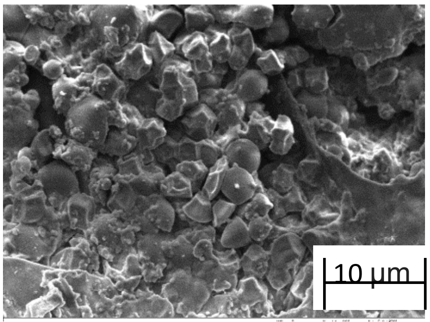


Figure 3.24 Differences in average hectolitre mass (HLM) values, obtained for all the HLM devices when samples were conditioned to different moisture levels (*ca.*14, 16 & 18%), determined with repeated analysis of variance (RANOVA). Different letters indicate significant differences obtained from Fisher least significant difference (LSD) post-hoc analysis. Vertical bars denote 0.95 confidence intervals.

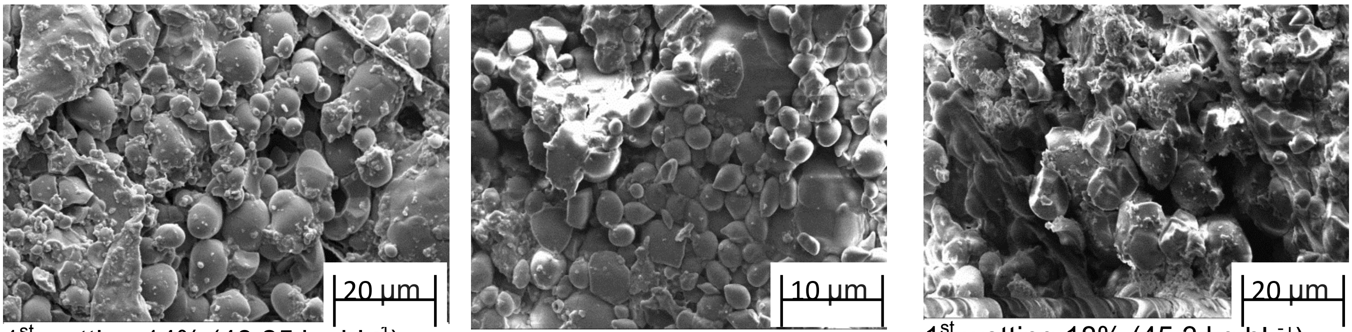
Experiment 9: effect of consecutive wetting and drying cycles on the microstructure of oats using scanning electron microscopy

The SEM micrographs (Figs. **3.**25-3.28) showed that in general the oat starch granules were polyhedral and irregularly shaped. Figs. 3.25 and 3.26 show the starch granules embedded in the protein matrix at 1000x magnificatioin closer to the edge of the kernel and in the center of the kernel, respectively. The same migrographs are shown at 3000x magnification in Figs. 3.27 and 3.28. Comparisons of the oat microstructures at different moisture contents revealed that the size of starch granules was affected. Overall the majority of starch granules became swollen and increased in size with increase in moisture content (first wetting). Swelling of the starch granules was characterised by a decrease in HLM for the sample, i.e. a significant decrease from 50.43 to 43.5 kg.hL^{-1} as the moisture increased from *ca*. 10.98 to *ca*.18% moisture content (Fig. 3.25). This indicates that swollen starch granules contribute to the increase in kernel volume (but not density) and thus lower HLM. Increase in size of the granules could more clearly be seen in the endosperm closer to the edge of the kernel at 3000x mangnification (Fig. 3.27). Shrinkage of starch granules was observed after the drying cycle. This could clearly be seen for the samples which were conditioned to *ca.* 14% moisture content and dried back to *ca*. 10%

(Fig. 3.27). As expected, the granules swelled again during the second wetting (after the drying cycle).



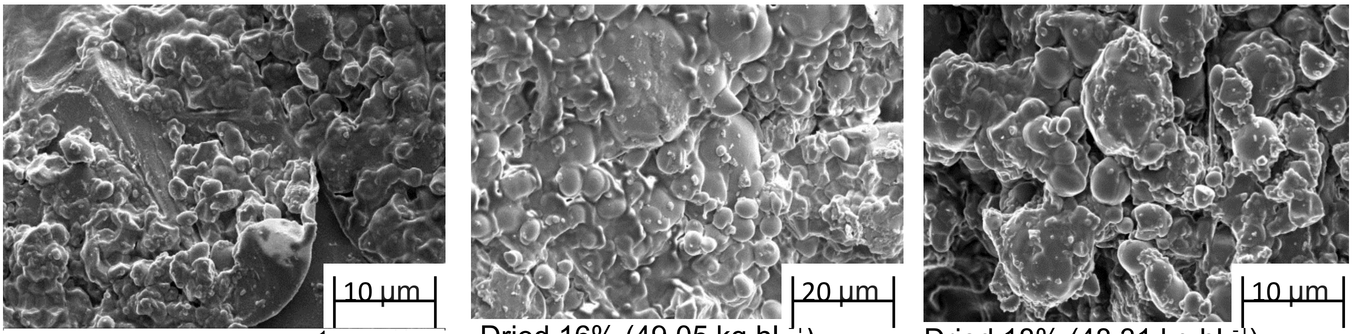
Control 10.98% (50.43 kg.hL^{-1})



1st wetting 14% (48.25 kg.hL^{-1})

1st wetting 16% (47.55 kg.hL^{-1})

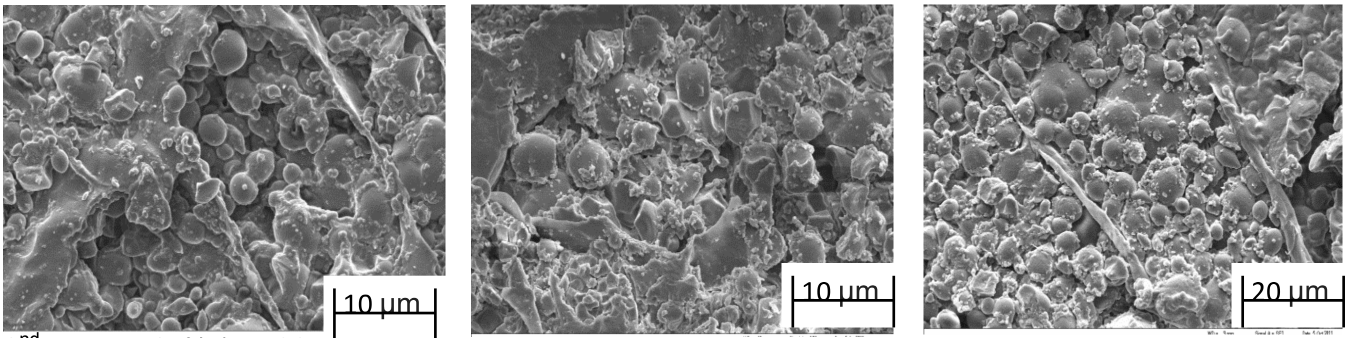
1st wetting 18% (45.3 kg.hL^{-1})



Dried 14% (49.78 kg.hL^{-1})

Dried 16% (49.05 kg.hL^{-1})

Dried 18% (48.31 kg.hL^{-1})

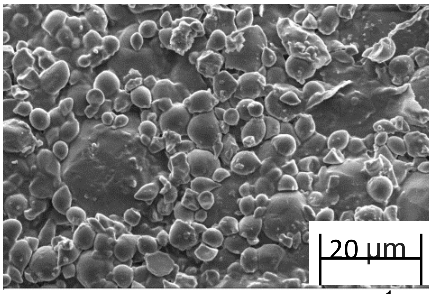


2nd wetting 14% (47.32 kg.hL^{-1})

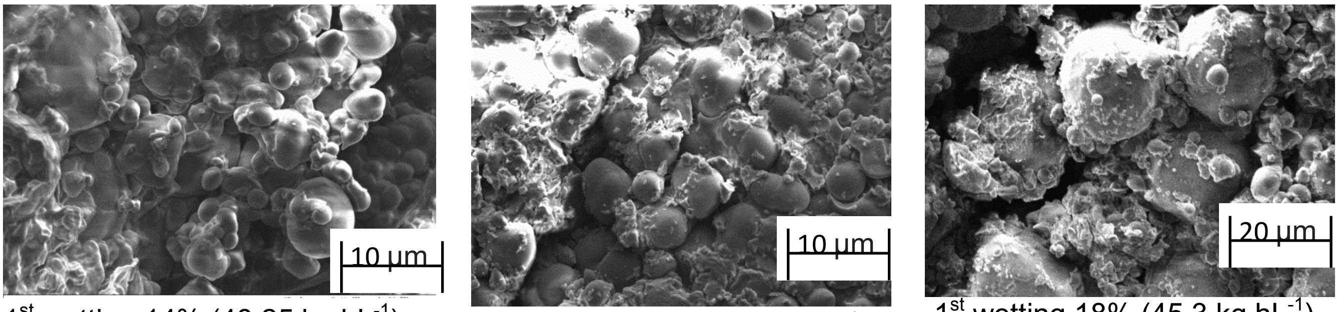
2nd wetting 16% (46.05 kg.hL^{-1})

2nd wetting 18% (44.0 kg.hL^{-1})

Figure 3.25 Scanning electron micrographs of oat kernels: edge of the endosperm; 1000X at different moisture treatments.



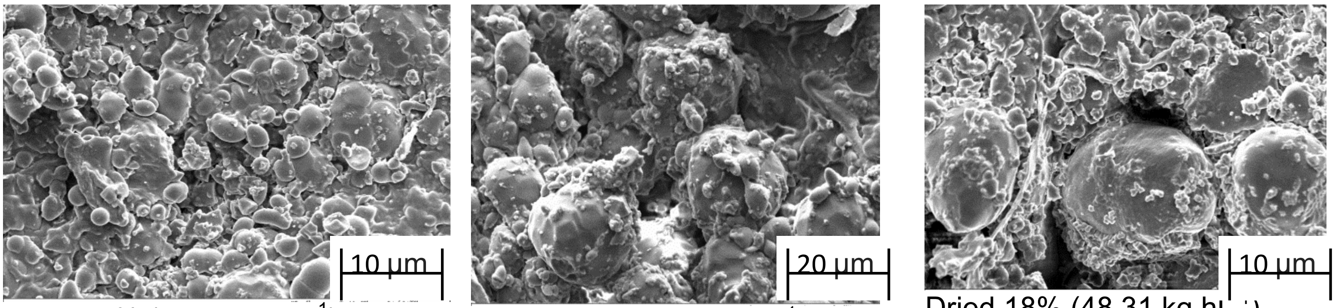
Control 10.98% (50.43 kg.hL^{-1})



1st wetting 14% (48.25 kg.hL^{-1})

1st wetting 16% (47.55 kg.hL^{-1})

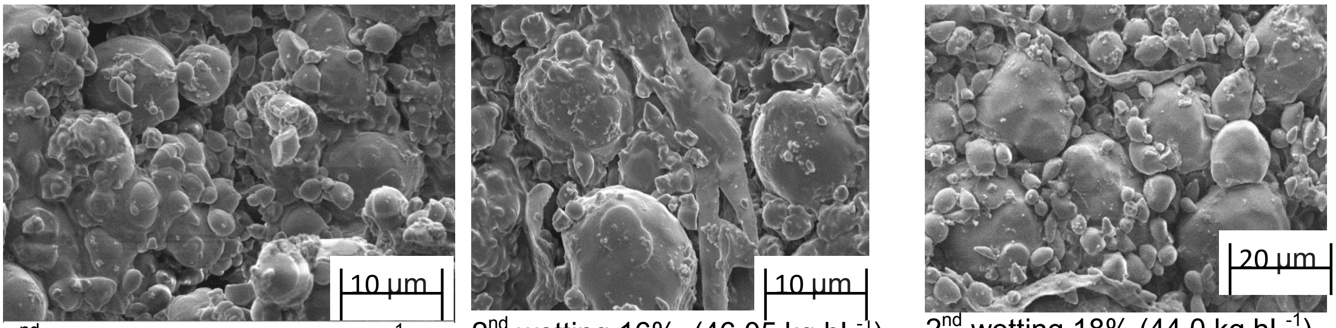
1st wetting 18% (45.3 kg.hL^{-1})



Dried 14% (49.78 kg.hL^{-1})

Dried 16% (49.05 kg.hL^{-1})

Dried 18% (48.31 kg.hL^{-1})

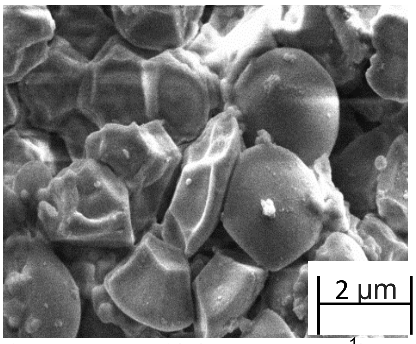


2nd wetting 14% (47.32 kg.hL^{-1})

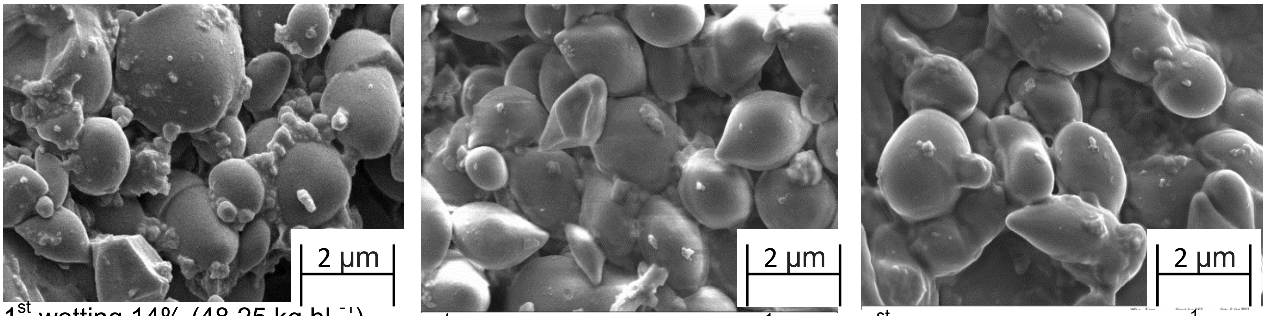
2nd wetting 16% (46.05 kg.hL^{-1})

2nd wetting 18% (44.0 kg.hL^{-1})

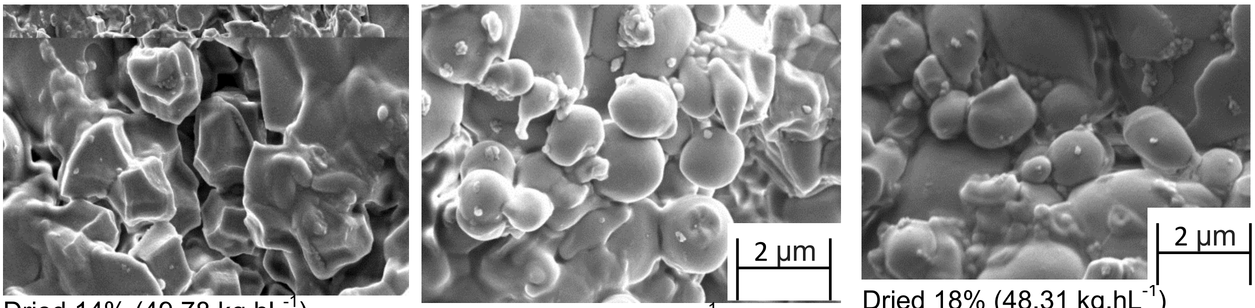
Figure 3.26 Scanning electron micrographs of oat kernels: center of the endosperm; 1000X at different moisture treatments.



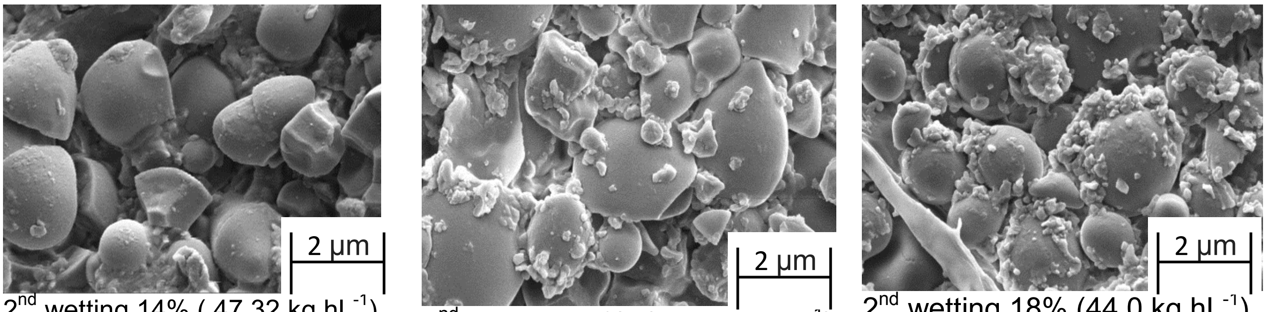
Control 10.98% (50.43 kg.hL^{-1})



1st wetting 14% (48.25 kg.hL^{-1}) 1st wetting 16% (47.55 kg.hL^{-1}) 1st wetting 18% (45.3 kg.hL^{-1})

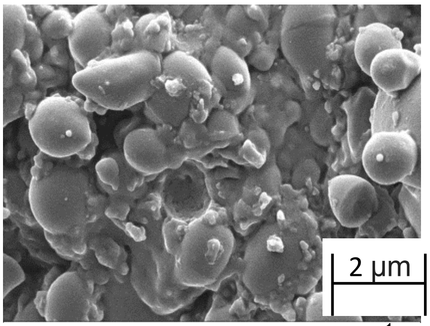


Dried 14% (49.78 kg.hL^{-1}) Dried 16% (49.05 kg.hL^{-1}) Dried 18% (48.31 kg.hL^{-1})

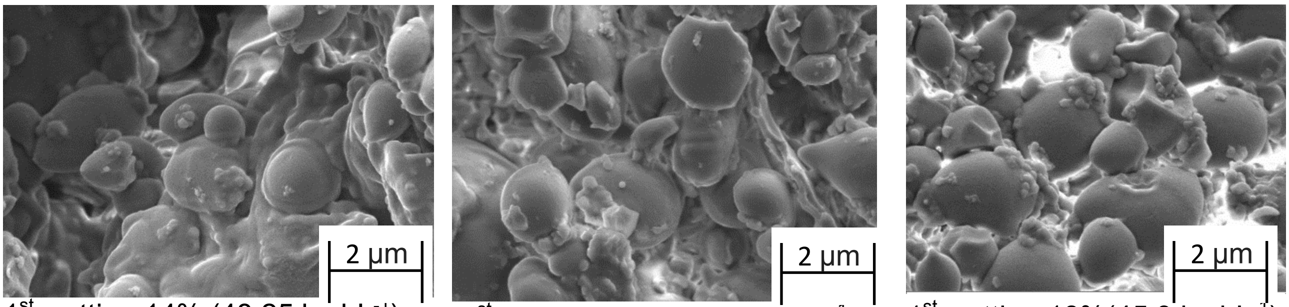


2nd wetting 14% (47.32 kg.hL^{-1}) 2nd wetting 16% (46.05 kg.hL^{-1}) 2nd wetting 18% (44.0 kg.hL^{-1})

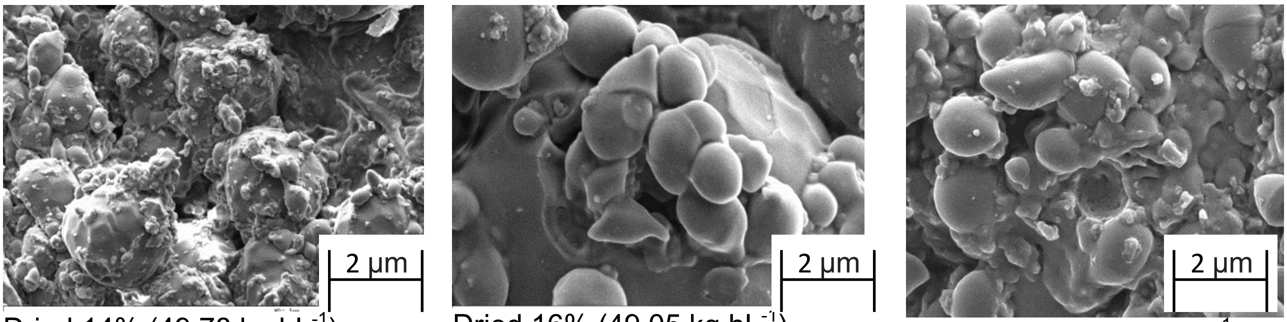
Figure 3.27 Scanning electron micrographs of oat kernels: edge of the endosperm; 3000X at different moisture treatments.



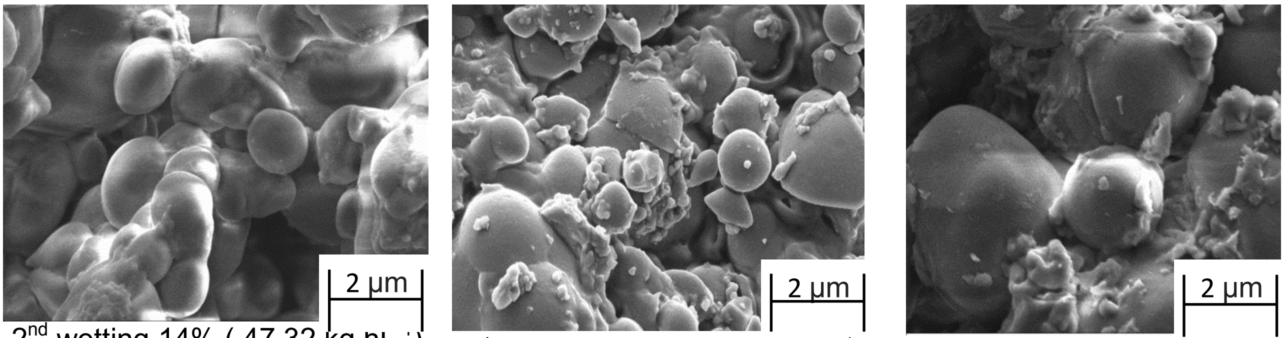
Control 10.98% (50.43 kg.hL^{-1})



1st wetting 14% (48.25 kg.hL^{-1}) 1st wetting 16% (47.55 kg.hL^{-1}) 1st wetting 18%(45.3 kg.hL^{-1})



Dried 14% (49.78 kg.hL^{-1}) Dried 16% (49.05 kg.hL^{-1}) Dried 18% (48.31 kg.hL^{-1})



2nd wetting 14% (47.32 kg.hL^{-1}) 2nd wetting 16% (46.05 kg.hL^{-1}) 2nd wetting 18% (44.0 kg.hL^{-1})

Figure 3.28 Scanning electron micrographs of oat kernel: center of the endosperm; 3000X at different moisture treatments.

Discussion

When one oat sample was used, there were no significant differences (*P*>0.05) between the German, Canadian, Australian, British and French devices. Even though the samples were mixed between repetitions, highly repeatable HLM values were obtained within each device (Appendix 2: Table 1). This result however would have been influenced by sample effect because only one oat sample was used. It is therefore important that samples meant for HLM measurements are truly representative and have not been damaged or changed during transportation or storage.

The results from the variation in repeatability within and variation between the HLM devices using single work samples further confirmed repeatable HLM measurements. There were no statistical differences (*P*>0.05) between the ten consecutive HLM measurements for any of the samples with any of the respective devices. Possible reasons for this could be: 1) there was no mixing of respective samples between repetitions; and 2) single work samples being used resulted in very little variation within each sample. This indicates that HLM values did not change significantly with increasing number of measurements on the same sample. This confirms similar results from a study done on wheat which showed that measuring the same sample repeatedly does not seem to have a significant effect on the HLM value of a sample (Engelbrecht, 2007). Thus the same oat sample could reasonably be used a number of times to do HLM determinations.

The RANOVA results for single work samples (Fig. 3.12) showed no statistical differences between the three sub-samples. These repeatable HLM measurements between sub-samples confirmed efficient sample preparation in terms of rubbing and sampling. Lack of variation also showed that samples were efficiently mixed and divided into sub-samples with a Boerner Divider. The Boerner Divider has been described as one of the best mixing and dividing equipment ideal for cereal related studies (Altuntas & Yıldız, 2007). The analysis of variance in HLM values was done using single work samples for ten respective oat samples. The ICC agreement was determined to evaluate differences in HLM measurements in terms of actual values between respective devices (Table 3.4). Overall, a high ICC agreement (0.88) and consistency value (0.99) were obtained between respective HLM devices indicating high correlation between the respective devices. The ICC agreement value observed in this study was slightly higher than ICC agreement results found by other researchers, i.e. overall ICC agreement (0.52) and ICC consistency (0.94) for mixed wheat (Manley *et al.*, 2009) and for maize (ICC agreement (0.52); ICC consistency (0.99)) (Engelbrecht, 2007).

Two SA devices currently being used commercially for HLM measurements of oats were evaluated compared to two German devices. The two SA devices did not differ significantly ($P>0.05$) from each other. Similarly, there was no statistical difference between the two German devices. Because only two South African devices were used in this investigation, one can not assume that similar results could be obtained when a number of these devices are evaluated together. Thus, a number of SA devices still needed to be evaluated together when measuring HLM of oats. Results from a study done on wheat revealed that statistical differences ($P<0.05$) were reported between the HLM results obtained from some of the ten SA devices evaluated (Manley *et al.*, 2009).

It has been observed that the HLM values significantly increased ($P<0.05$) when oat samples were rubbed (Fig. 3.17). Increase in HLM values is accounted for by physical changes that grain undergo upon rubbing. Frequently the tips of the oat extend beyond the length of the groat. Rubbing breaks off these tippy hulls as well as the tricomes on the oat kernels. This results in improved packing. Previous studies have concluded that polishing or mechanically rubbing of oat grain shortens their hulls and this is associated with a higher packing efficiency and higher HLM values (Cutler, 1940; Doehlert *et al.*, 2006).

The unskilled operator measured significantly higher HLM values than the skilled and semi-skilled operator. The difference in operator results was most likely due to the way the unskilled operator conducted the HLM measurements. The unskilled operator had never performed any HLM measurements, therefore was more likely to affect the manner of pouring the grain in the test container. Unknowingly vibrating, shaking or taping the measuring containers would have caused compaction of the grain, thus resulting in falsely high HLM values. Operator effect on HLM of wheat was also pointed out by other researchers (Greenway *et al.*, 1971; Manley *et al.*, 2009). Generally, if the unskilled operator had some training beforehand, he would produce results in agreement to those of skilled operators (Greenway *et al.*, 1971).

There was no apparent relationship between HLM and protein content of oat samples. This is in line with results observed in durum wheat grown under a wide range of nitrogen fertilizer levels (Dexter *et al.*, 1987) with no relationship between HLM and protein content. Protein content can be either positively or negatively associated with HLM (Gaines, 1991). The decrease in HLM with increased protein content can be caused by factors such as environmental stress (Preston *et al.*, 1995) rather than a direct response to protein content.

The average HLM values were found to decrease as the moisture content of oat samples increased from *ca.* 10 to 18% moisture content (Fig. 3.21). These results are in agreement with previous studies which showed a significant decrease ($P<0.05$) in HLM values with increasing moisture content for wheat (Pushman, 1975; Lloyd *et al.*, 1999; Manley *et al.*, 2009), maize (Rankin, 2009) and faba bean (*Vicia faba L.*) (Altuntas & Yıldız, 2007). Changes in HLM values of oats when wetted to different moisture levels are linked to the changes in density and integrity of the kernel. Consecutive wetting and dry cycles loosen the pericarp layers of the grain. Kernels swell and do not contract back to their size on drying. Swelled kernels have more volume and this reduces the number of the grains that will fit in the test container.

The SEM micrographs clearly showed that oat starch granules swelled with increasing moisture content. Conversely, when the samples were dried, granules became smaller. It has been established conclusively that cereal starch granules shrinks when dried and swells again when moistened (Alsberg, 1938). During second wetting starch granules did not swell more as would be expected. It had been observed that even moderate drying reduces the power of cereal starch granules to swell (Alsberg, 1938) they do not, at least for some time, swell to the same size they would have reached had they not been dried. The decrease in HLM values with increased moisture content is thus accounted for by swelling of starch granules. Swollen starch granules increased the size or volume of the grain and reduced the number of grains fitting in the test container with subsequent reduction in HLM.

Conclusion

Variation between HLM devices were observed in this study. The analysis of variance (RANOVA) results have shown similar trends between average HLM values as obtained with different devices in all respective investigations. The SA device measured lower HLM values (apart from compared to the USA device), a trend that was reported in a previous study with wheat. Results obtained in this study revealed a need to re-evaluate HLM devices currently used in the South African oat industry. The increase in HLM values observed when oats were rubbed shows the importance of rubbing. The purpose of oat rubbing was not to obtain purposefully increased HLM values. However, it demonstrates how the HLM is affected as a result of morphological changes the oat kernel undergoes when rubbed. Therefore, rubbing is of commercial benefit to the South African oat industry and should be continued as part of the sample preparation of HLM measurements. It has been shown clearly that operators may produce different HLM measurements with the unskilled operator measuring significantly higher. The consistency and efficiency of operators could be improved through training and experience. The investigation on protein analysis confirmed that no clear relationship existed between HLM and protein content of oat samples. Consecutive wetting and drying cycles had a significant effect on the HLM determinations of oats. SEM results revealed that starch granules swell with increased moisture content. At high moisture contents starch granules contribute to the increase in size of the oat grain, a factor associated with low HLM.

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Chapter 4

Potential use of NIR hyperspectral imaging to distinguish between oat samples with different hectolitre mass (HLM) values

Chapter 4

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Abstract

Testing whole, unground cereal kernels is often required by plant breeders as they subsequently need to plant what they have selected. Near infrared (NIR) spectroscopy, being a non-destructive technique, offers this option to plant breeders. This study evaluated the use of NIR spectroscopy combined with digital imaging, i.e. NIR hyperspectral imaging, to distinguish between six oat samples differing in hectolitre mass (HLM). NIR spectroscopic differences were observed between the images of the two samples with the lowest and highest HLM values (49.35 and 60.2 kg.hL^{-1}). Fewer differences were observed in the NIR hyperspectral images of two samples differing less than 2.0 kg.hL^{-1}. Although mixed oat samples were used, these preliminary results established the possible use of NIR hyperspectral imaging in evaluating oat samples in terms of HLM and the potential to adopt NIR spectroscopy and combined with imaging for the assessment of grain in breeding trials.

Introduction

Oats are increasing in popularity as part of the human diet and being added either as whole or fractionated groat to many food products (Wang & White, 1994; Anderson *et al.*, 2009). The most important factor contributing to the increased demand for oat products is the recent health claims describing the nutritional value of oats (Masood *et al.*, 2008). The fiber component (β-glucan) and natural antioxidants are two oat components claimed to have possible health benefits (Wang & White, 1994).

Trading in oats requires quality measurements to be performed rapidly. Hectoliter mass (HLM) is a widely recognised quality grading specification because it is related to the soundness of the grain, and is often used as an index of milling potential (Doehlert, 2002). HLM measurements allow rapid and accurate determination of grain density. Moisture content, climate conditions, kernel size, density and packing factors affect HLM (Forsberg & Reeves, 1992; Marshal & Sorrells, 1992; Doehlert *et al.*, 2006). HLM can also be used as a silo management tool to optimise the use of storage space in the silo (Fox *et al.*, 2007). Cereal grains are often graded by a combination of visual inspection as well as using rheological and analytical instruments. Visual assessment of cereal grains can be subjective particularly when borderline samples are graded. Analytical work at the time of grain purchasing includes mainly testing for moisture and chemical composition while rheological measurements include evaluation of the physiochemical properties of the grain (Williams, 2010). Analyses of cereals by conventional analytical and/or rheological laboratory methods are often expensive and too time-consuming for practical applications.

Near infrared (NIR) spectroscopy has made it possible to routinely analyse materials that could not be analysed conveniently or economically before because of the restriction in time, expenses and sample size required (Li-chan *et al.*, 2010). NIR spectroscopy is a technique based on the information gathered from a substance when it absorbs NIR radiation (Woodcock *et al.*, 2008). It has been used for detection, classification and quantification in a variety of sorting and/or quality control applications. NIR spectroscopy is a flexible, robust, environmental friendly analytical tool used in most industrial disciplines (agriculture, food, pharmaceuticals, petrochemicals and environment).

Recently conventional NIR spectroscopy has successfully been used to predict bulk density (HLM) in whole brown rice (Himmersbach, 2010). NIR could be useful in the screening of large numbers of oat breeding lines for several important quality factors.

NIR hyperspectral imaging, which is a technique that combines conventional NIR spectroscopy and digital imaging, has an added spatial dimension (Geladi *et al.*, 2004; Burger, 2006). NIR hyperspectral imaging allows the efficient analysis of small amounts of sample, which is often all that is available in breeding trials, due to a spectrum being collected for each pixel. The latter is due to added spatial dimension (Li-chan *et al.*, 2010). The aim of this study was to examine the potential use of NIR hyperspectral imaging to distinguish between oat samples with different HLM values. The successful outcome of this study will be useful to South African oat breeders if HLM is used as a deciding characteristic when selecting future cultivars. This is because NIR spectroscopy offers the option to plant breeders to test whole, unground kernels non-destructively and the analysed grain can subsequently still be planted.

Materials and methods

Samples and sample preparation

Six oat samples (50 g each) with HLM values ranging from 49.5 to 60.2 kg.hL^{-1} were used. The HLM of each sample was confirmed using the German HLM device as described earlier (Chapter 3, page 41). Twenty kernels were randomly selected from each sample for subsequent NIR hyperspectral imaging.

NIR hyperspectral imaging

A SisuCHEMA NIR hyperspectral imaging system (Specim, Spectral Imaging Ltd., Oulu, Finland) was used to collect the images. This is a pushbroom system which works in reflectance mode. An objective with a 5 cm wide field-of-view was used, hence each pixel/spectrum represented a 150 × 150 µm area. ChemaDAQ (version 3.62.183, Specim, Spectral Imaging Ltd., Oulu, Finland) was used to collect data from the 1000-2500 nm spectral range in 6.3 nm intervals. ChemaDAQ was also use to control the sample stage and to automatically correct the image data using internal dark and external white reference standards. Each sample set of 20 kernels was imaged individually except for the two samples used to test repeatability of the measurements. The latter two samples were imaged twice (repacked and refocused before second image was taken) to confirm that the differences observed in the images were not due to differences caused by focusing.

Evince (version 2.4.0 Umbio AB, Umeå, Sweden) was used for all image processing and data analyses. Principal component analysis (PCA) with nine components was used on mean-centered data to clean the images. This involved the utilisation of PCA score plots and score images interactively to identify and classify unwanted pixels such as outliers, background, dead pixels, shading errors and edge effects. The unwanted pixels were removed. Standard normal variate (SNV) transformation, a mathematical transformation that removes multiplicative interferences such as scattering and particle size differences was applied and PCA scores and loadings were recalculated. PCA score images, score plots and principal component (PC) loading line plots were studied.

Results and discussion

The PCA score plots and score images were investigated to determine differences between the two samples with the lowest (49.35 kg.h.L^{-1}) and highest (60.2 kg.h.L^{-1}) HLM values. PCA score plot of PC1 (59.9%) vs. PC6 (1.05%) (Fig. 4.1a) revealed two clusters. The location of the pixels in the score image (Fig. 4.1b) confirmed that pixels on the negative side of PC1 represent the 60.2 kg.hL^{-1} sample and those on the positive side of PC1 represent the 49.35 kg.h.L^{-1} sample. The classification plot (Fig. 4.2a) and image (Fig. 4.2b) allowed a clear visualisation between these two samples.

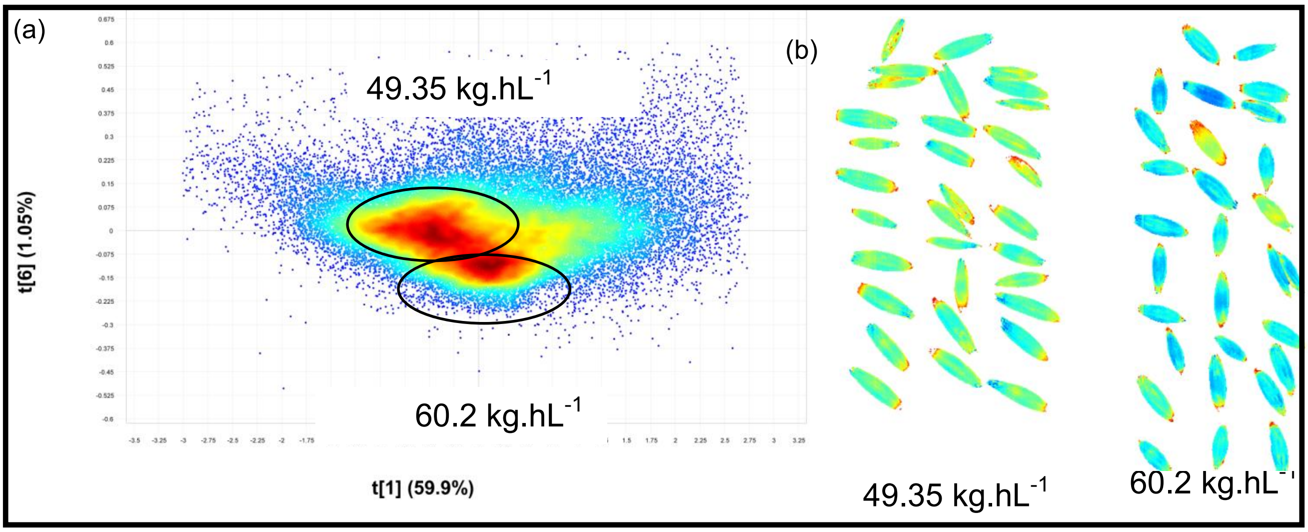

Figure 4.1 (a) PCA score plot of PC1 vs. PC6 and (b) PC6 score image showing differences between two oat samples with HLM values of 49.35 and 60.2 kg.hL^{-1}, respectively.

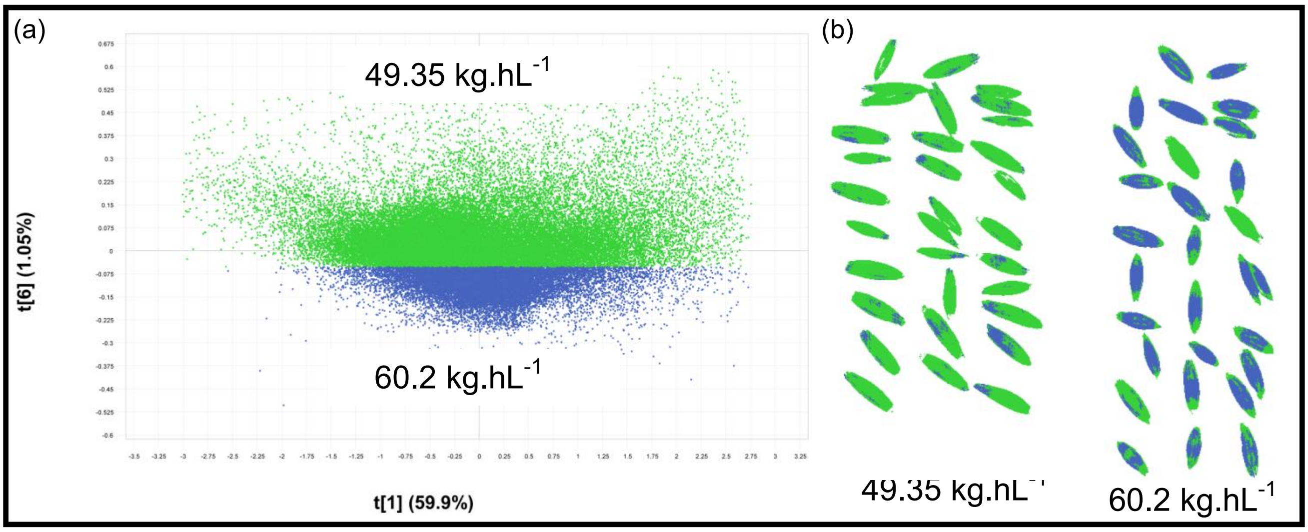

Figure 4.2 (a) Classification plot of PC1 vs. PC6 and (b) classification image of PC6 (green = 49.35 kg.hL^{-1} and blue = 60.2 kg.hL^{-1}).

Morphologically, the 60.2 kg.hL^{-1} sample was more round and plumper than the 49.35 kg.hL^{-1} sample. The difference in shape of the two samples is one of the reasons why the difference in HLM was only observed in PC6. This phenomenon is explained in more detail in a paper by Manley *et al*. (2011).

Loading line plots (Fig.4.3) were investigated to determine which variables contributed mostly to the respective PC. There were no prominent peaks in the PC1 loading line plot (Fig 4.3a). The PC2 loading line plot resulted in two prominent peaks positively weighted: 1524 nm (O-H stretching first overtone intra-molecular H-bond); 2092 nm (O-H stretching +

OH deformation). These two peaks were both related to starch. Two peaks that were negatively weighted appeared at 124 nm (related to water) and 2266 nm (O-H stretching + OH deformation; related to starch). The spectral difference between the two samples were confirmed by plotting their average spectra before (Fig. 4.4a) and after SNV transformation (Fig. 4.4b).

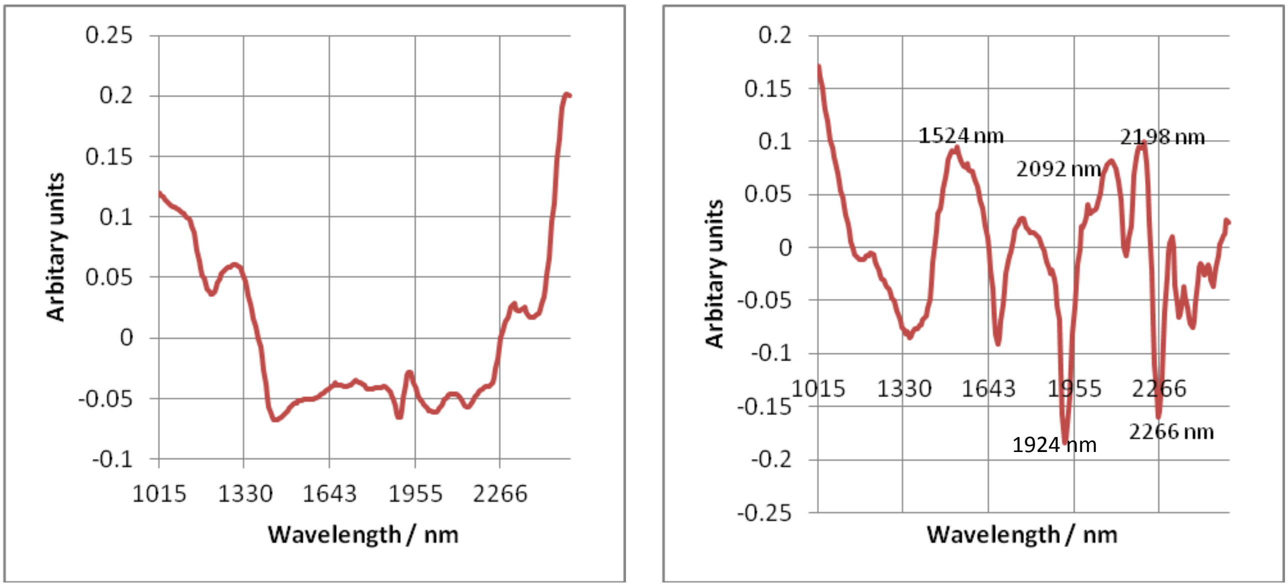
(a) (b)

Figure 4.3 (a) PC1 loading line plot and (b) PC6 loading line plot for the 49.35 and 60.2 kg.hL^{-1} samples.

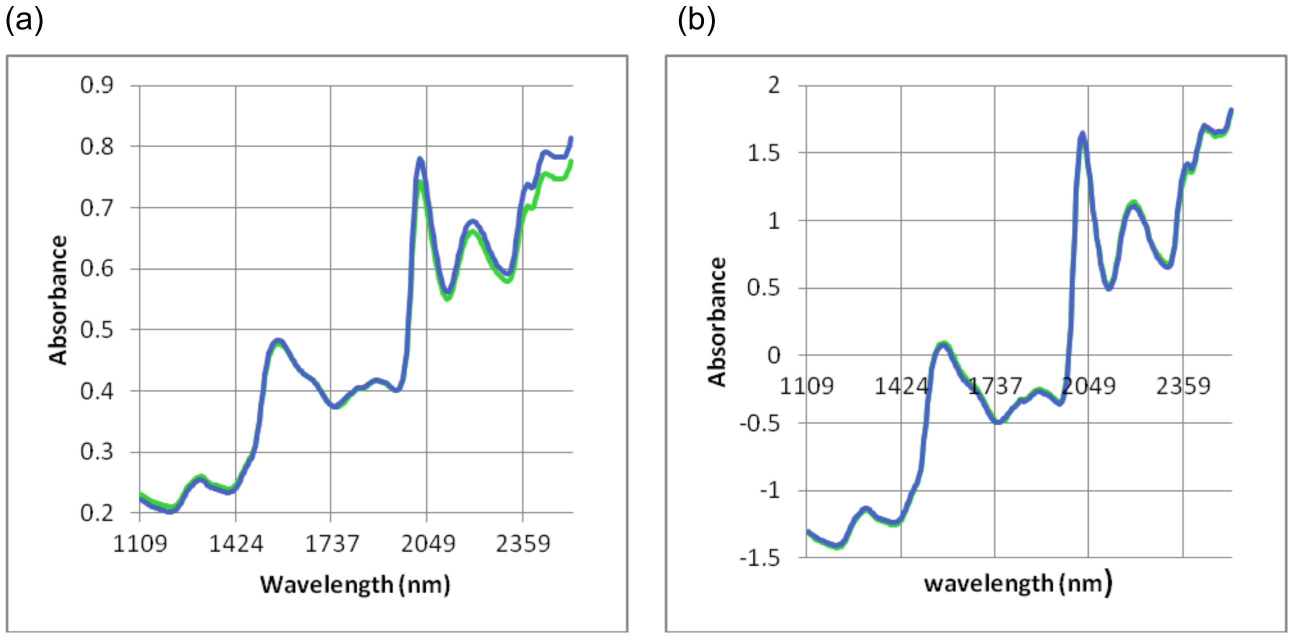

Figure 4.4 (a) Raw spectra obtained after averaging the hyperspectral images of the 49.35 kg.hL^{-1} (green) and 60.2 kg.hL^{-1} (blue) samples and (b) the average spectra after standard normal variate (SNV) transformation.

It was subsequently also attempted to distinguish between two oat samples with a smaller difference in HLM (5.2 kg.hL^{-1}). Image analysis of these two samples (Fig. 4.5) produced similar results to those obtained when samples with a difference of 10.85 kg.hL^{-1} were compared. Two clusters were observed on the positive and negative side of PC6, respectively (Fig. 4.5a). The cluster with negative score values was associated with the 55.75 kg.hL^{-1} sample. The classification plot (Fig. 4.6a) and image (Fig.4.6b) allowed more clear visualisation that it is possible to distinguish between these two samples.

The PC6 loading line plot revealed three peaks (Fig. 4.7b) of which two were positively weighted, i.e. 1918 nm (related to starch) and 2260 nm (2x N-H symmetric + amide III; related to protein). The 2111 nm peak (N-H symmetric + amide + III; related to protein) was negatively weighted. The average raw (Fig. 4.8a) and SNV transformed (Fig. 4.8b) spectra confirmes the differences between these two samples.

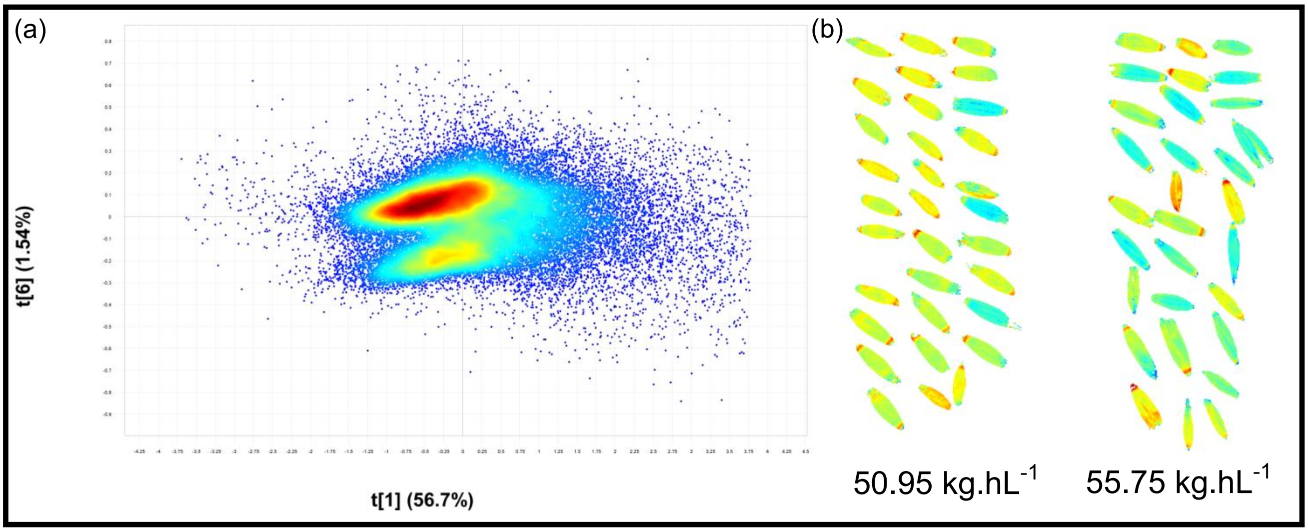

Figure 4.5 (a) PCA score plot of PC1 vs. PC 6 and (b) PC6 score image showing differences between two oat samples with HLM values of 50.95 and 55.75 kg.hL^{-1}, respectively.

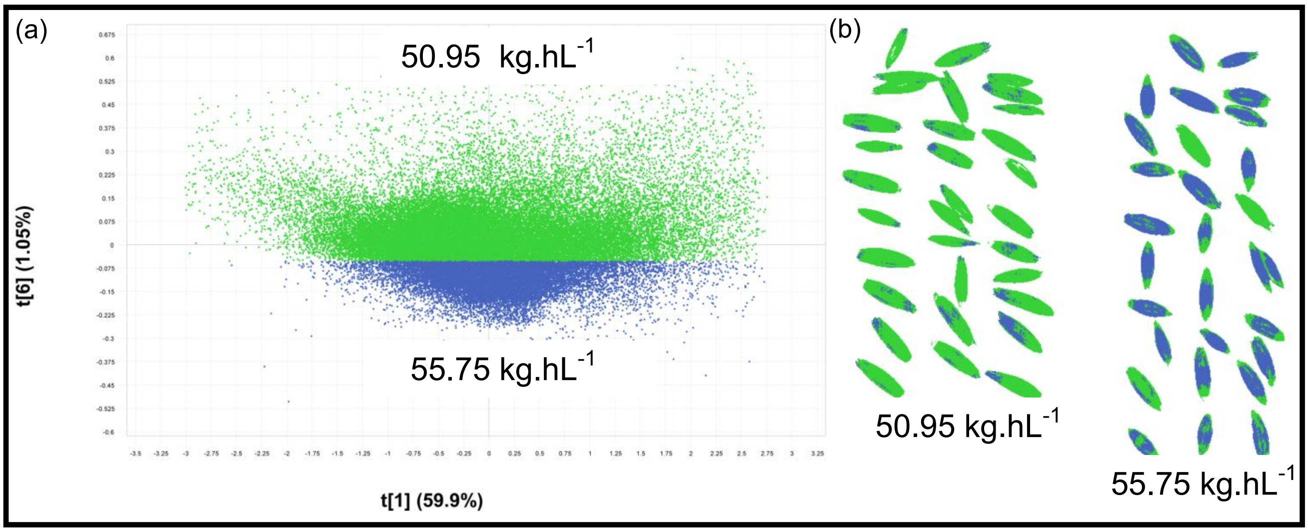

Figure 4.6 (a) Classification plot of PC1 vs. PC6 and (b) classification image of PC 6 (green = 50.95 kg.hL^{-1}and blue = 55.75kg.hL^{-1}).

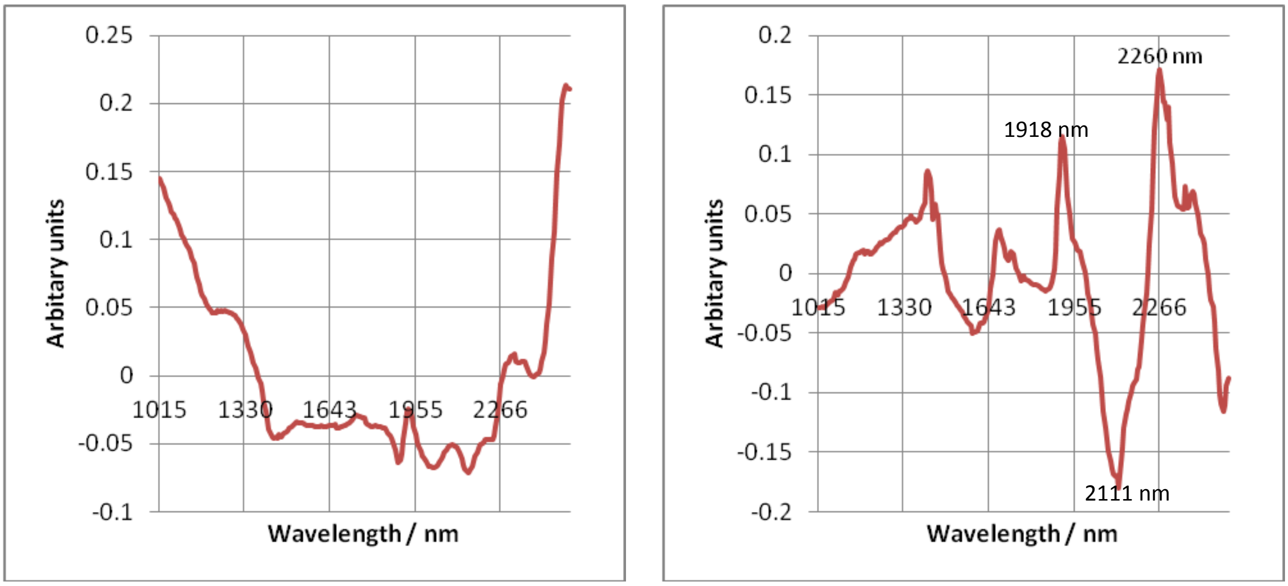

Figure 4.7 (a) PC1 loading line plot and (b) PC6 loading line plot for the 50.95 and 55.75 kg.hL^{-1} samples.

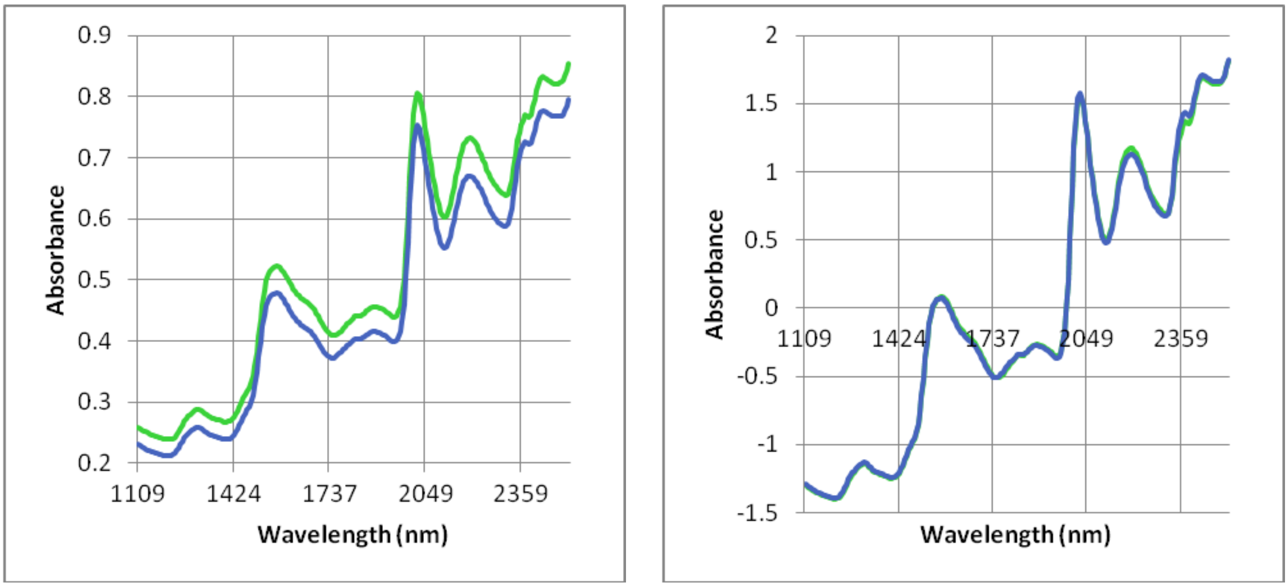

Figure 4.8 (a) Raw spectra obtained after averaging the hyperspectral images of the 50.95 kg.hL^{-1} (green) and 55.75 kg.hL^{-1} (blue) samples and (b) the average spectra after standard normal variate (SNV) transformation.

NIR hyperspectral image analysis was finally performed on two samples with only a 2.0 kg.hL^{-1} difference in HLM (Fig. 4.9). There were no distinct clusters in any combination of PC1 to PC9 score plots. The PCA score plot of PC1 vs. PC6 is shown in Fig. 4.9a and the PCA score image (Fig. 4.9a) showed no differences that could allow classification between these two samples (both samples characterised by light blue-green colour). When a classification plot and image was constructed based on the results obtained earlier, both samples appeared to have equal representation of green and blue in the classification image (Fig. 4.10b). The similarity in spectral properties (Fig. 3.5b) is an indication of certain similarity in chemical and/or physical (e.g. density) properties. These two samples also had more similar crude protein contents, i.e. the 58.45 kg.hL^{-1} sample had a protein content of 7.72% (12% mb) and the 60.2 kg.hL^{-1} a value of 8.73% (12% mb).

Loading line plots were interpreted as before. No major peaks were observed in PC1 (Fig.4.11a). Three prominent peaks were observed in the PC6 loading line plot (Fig. 4.11b) with two wavelengths negatively weighted, i.e. 1937 nm (O-H stretching + O-H deformation; related to water) and 2136 nm (N-H stretching + C=O stretching; related to protein). A positively weighted absorption peak appeared at 1868 nm (related to starch). The similarity between these two samples can also be seen in the average raw (Fig 4.12a) and SNV transformed spectra.

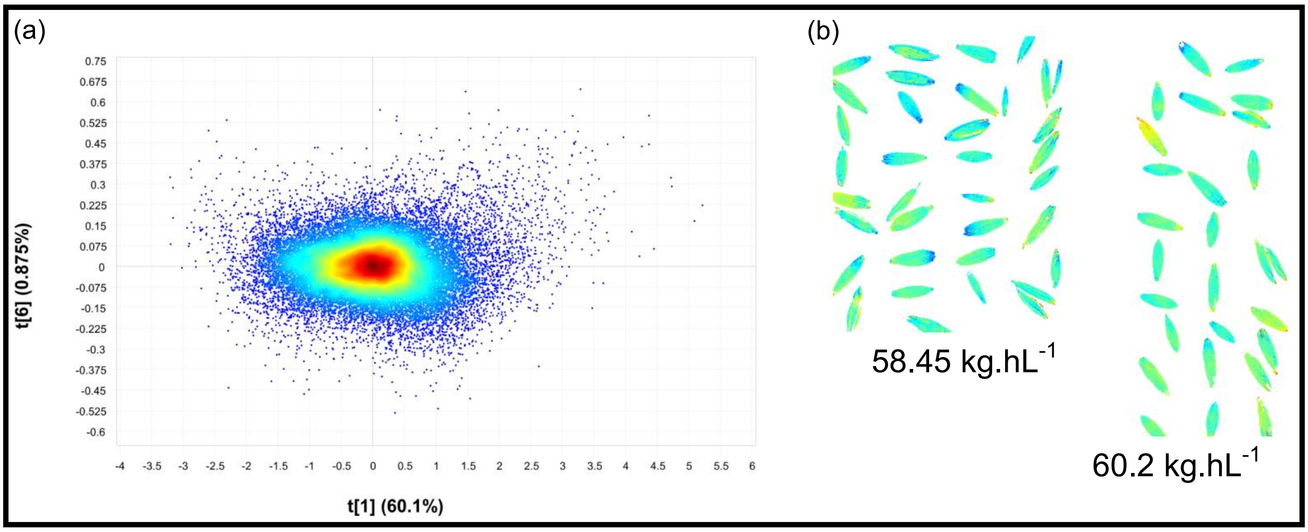

Figure 4.9 (a) PCA score plot of PC1 vs. PC 6 and (b) PC6 score image showing differences between the two oat samples with HLM values of 58.45 and 60.2 kg.hL^{-1}, respectively.

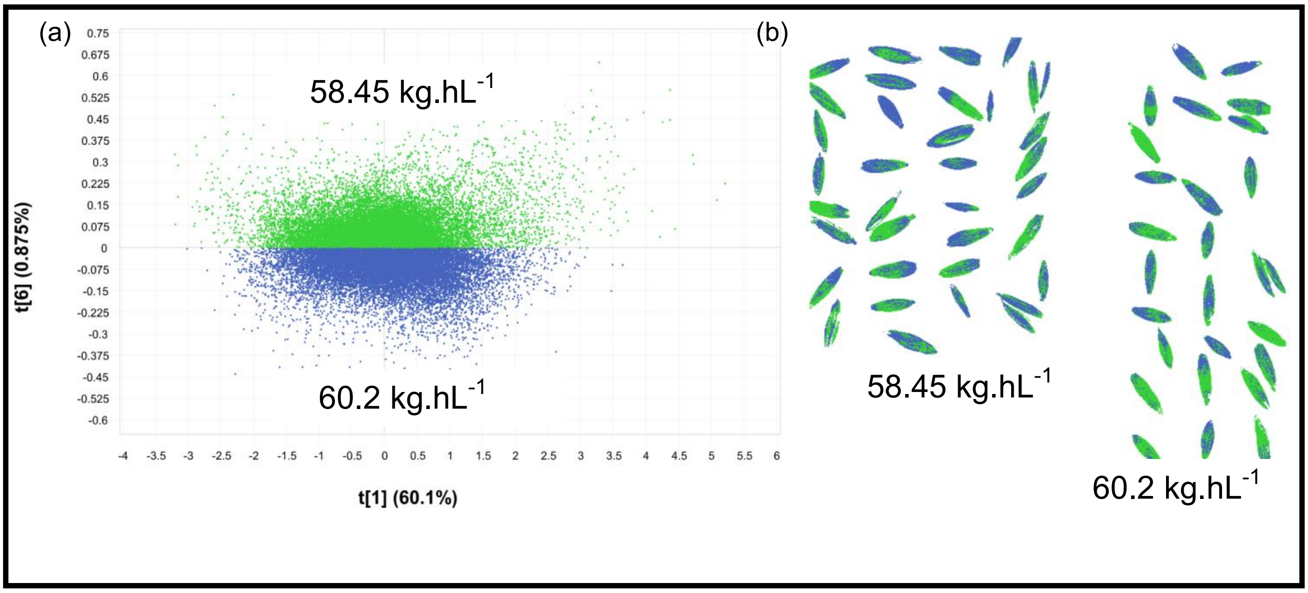

Figure 4.10 (a) Classification plot of PC1 vs. PC 6 and (b) classification image of PC6 showing similarities between the two oat samples with HLM values of 58.45 and 60.2 kg.hL^{-1}, respectively.

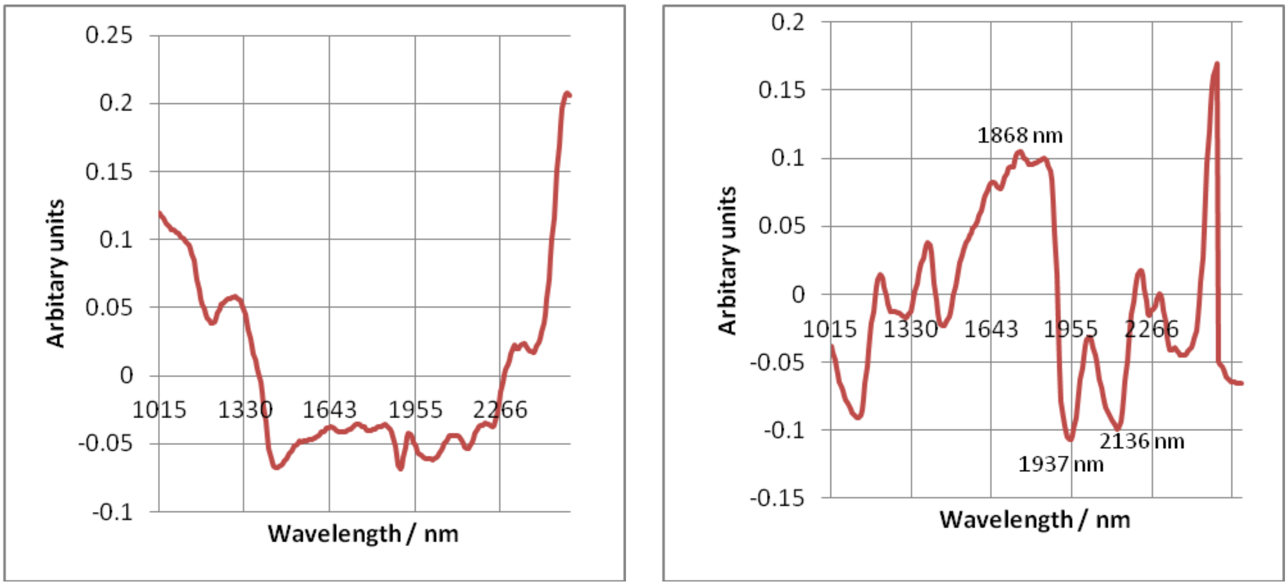

Figure 4.11 (a) PC1 loading line plot and (b) PC6 loading line plot for the 58.45 and 60.2 kg.hL^{-1} samples.

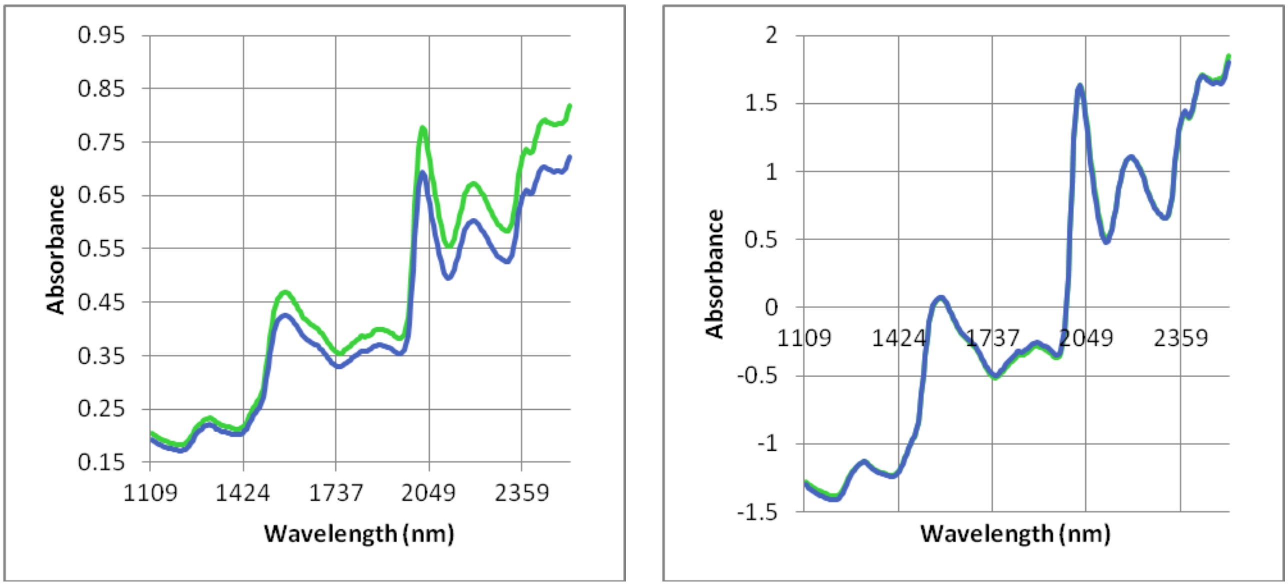

Figure 4.12 (a) Raw spectra obtained after averaging the hyperspectral images of the 58.45 kg.hL^{-1} (green) and 60.2 kg.hL^{-1} samples (blue) and (b) the average spectra after standard normal variate (SNV) transformation.

To confirm the validity of the earlier results two oat samples, one with a low (49.35 kg.hL^{-1}) and another with a high (60.2 kg.hL^{-1}) HLM value were imaged twice. For the second image the samples were removed from the samples stage, repacked and imaged again after refocusing. After PCA was applied it was clear that the pixels on the positive side of PC6 in the score plot (Fig. 4.13a) were again associated with the 49.35 kg.hL^{-1} sample and those on the negative side associated with the 60.2 kg.hL^{-1} sample. The similarity between the images before and after repacking can also be seen in the PCA score image of PC6 (Fig. 4.13b). Thus repacking and refocusing did not have a significant effect on the spectral data obtained. The difference seen between the earlier samples that were imaged separately were thus not because of differences in focussing, but chemical or scattering differences.

The NIR hyperspectral image differences between the two samples can be visualized in the classification plot and image (Figs 4.14). No differences because of repacking were observed.

The third cluster observed in this case in the PC1 vs. PC6 plots (Fig. 4.13a) is shown in the classification score plot (Fig. 4.14a.) and image (Fig 4.14b) to explain the shape of the kernels (Manley et al. 2011). The red class thus represents edge effects where as the green and blue indicate spectral differences between the two high and low HLM samples, respectively. No prominent peaks were observed in PC1 loading line plot (Fig.4.15a). Three peaks were prominent in PC6 loading line plot; two positively weighted at 1443 nm (related to starch) and 2260 nm (representing protein), respectively (Osborne *et al.*, 1993). The 2080 nm peak (related to starch) was negatively weighted. There was thus little difference in the average raw spectra of the two samples before and after repacking and refocusing (Fig. 4.17), confirming the difference seen between the earlier samples were due to chemical or density differences.

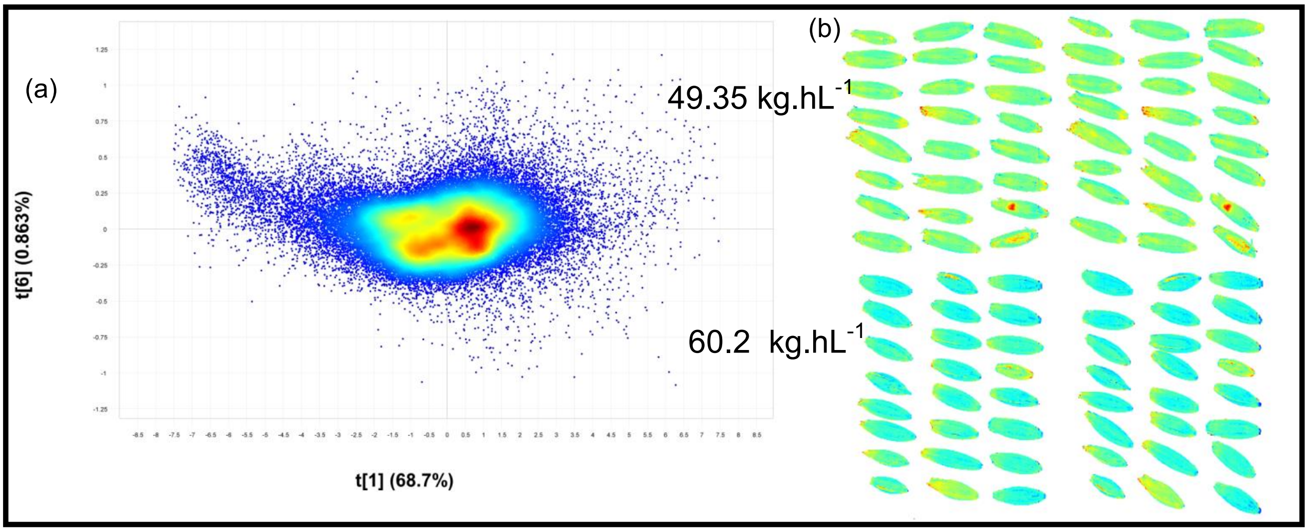

Figure 4.13 (a) PCA score plot of PC1 vs. PC 6 and (b) PC6 score image showing differences between two oat samples (HLM values of 49.35 and 60.2 kg.hL^{-1}) top and bottom and no differences within each sample after repacking and refocusing (left and right).

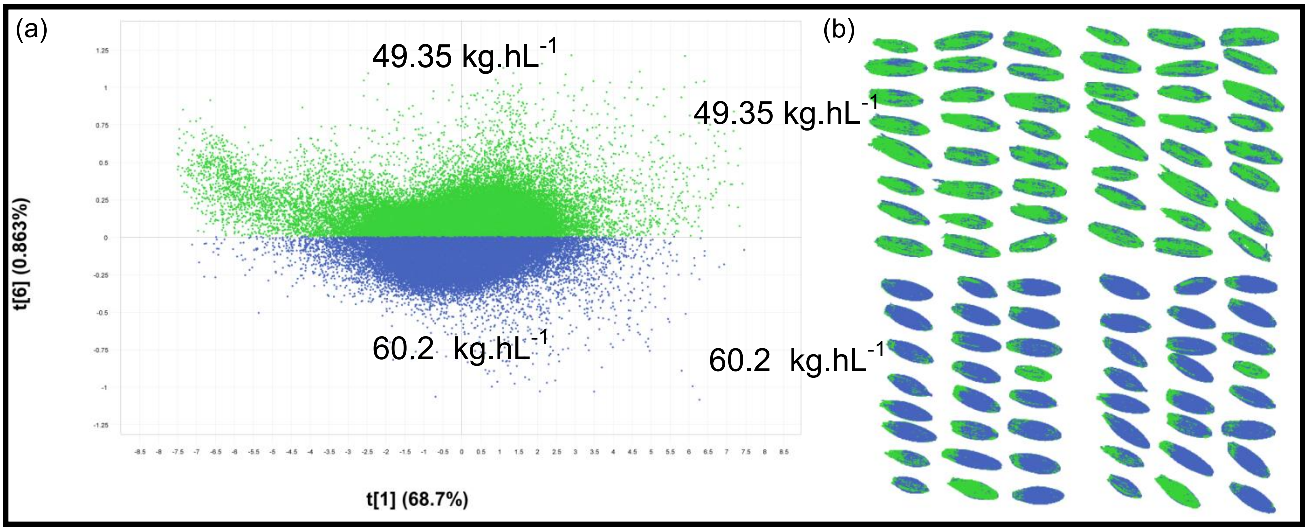

Figure 4.14 (a) Classification plot of PC1 vs. PC6 and (b) classification image of PC6 (green = 49.35 kg.hL^{-1} and blue = 60.2 kg.hL^{-1}). There are no differences within each sample after repacking and refocusing (left and right).

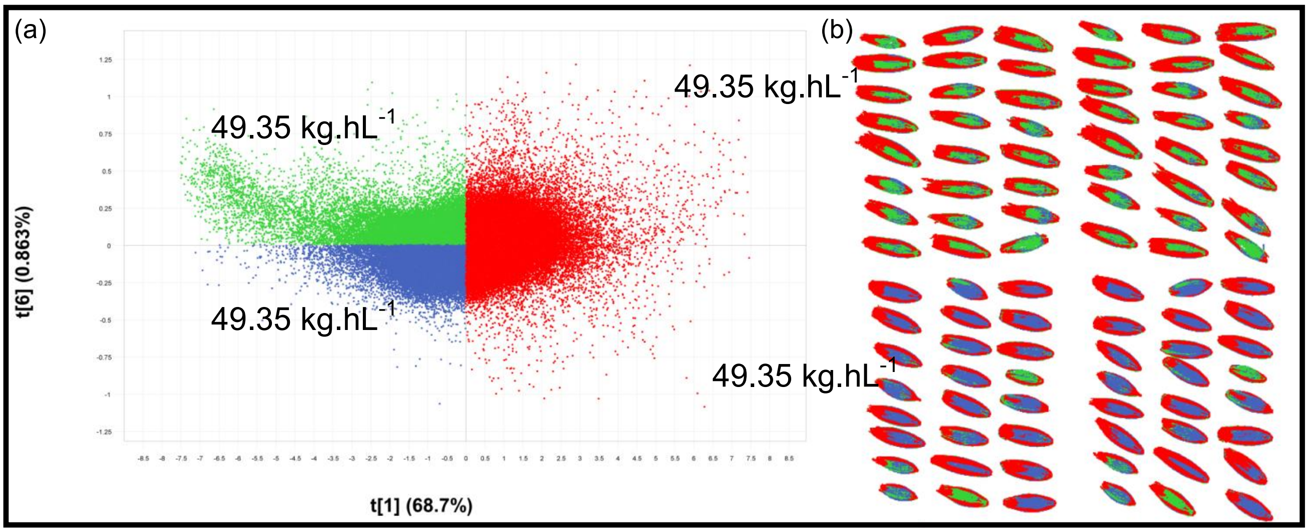

Figure 4.15 (a) Classification plot of PC1 vs. PC6; (b) classification image showing differences between 49.35 kg.hL^{-1} and 60.2 kg.hL^{-1} samples (green = 49.35 kg.hL^{-1}; blue = 60.2 kg.hL^{-1}; red = edge of the kernels).

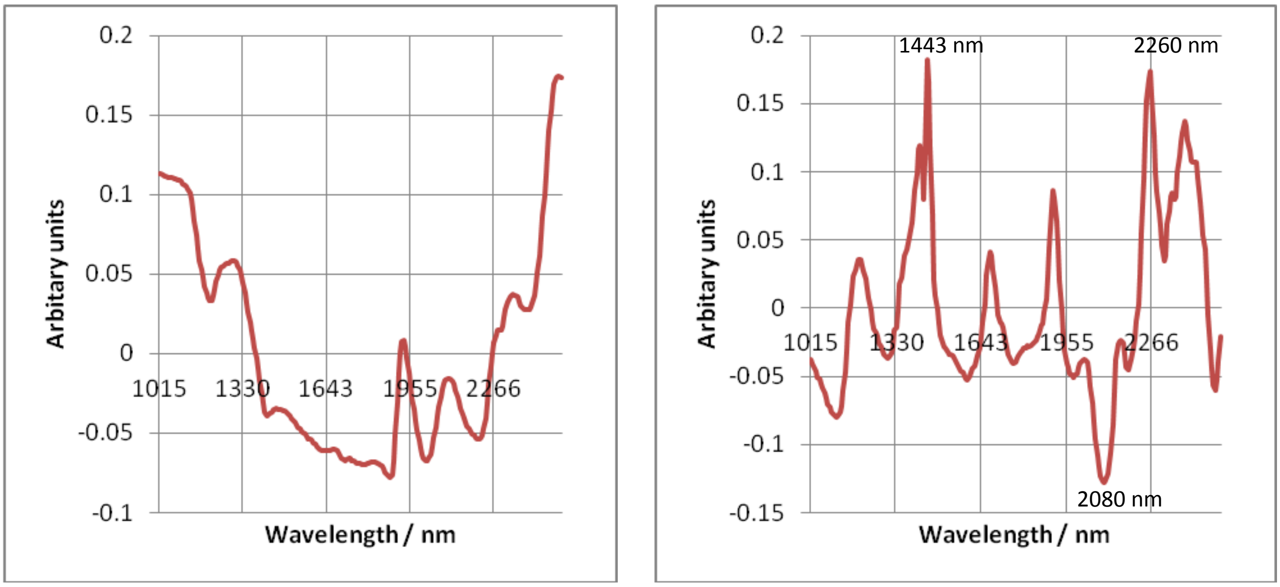

Figure 4.16 (a) PC1 loading line plot; (b) PC6 loading line plot for the 49.35 and 60.2 kg.hL^{-1} sample images.

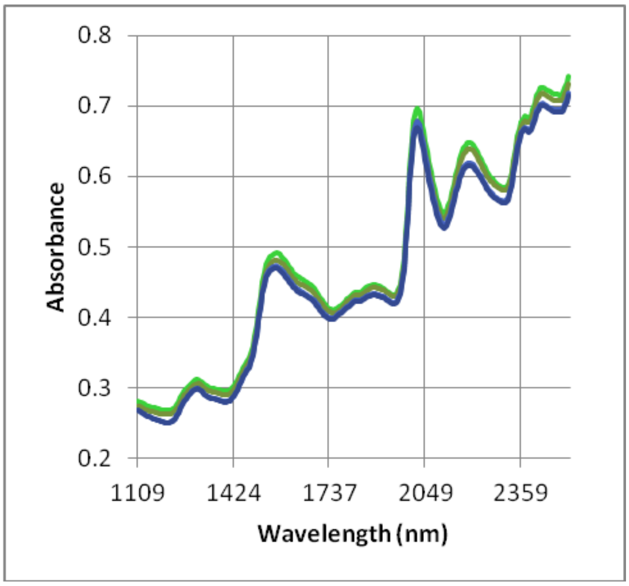

Figure 4.17 (a) Raw spectra obtained after averaging the hyperspectral images of the 58.45 kg.hL^{-1} (green= before repacking; light green = after repacking and refocusing) and 60.2 kg.hL^{-1} (blue = before repacking; dark blue = after repacking and refocusing) samples.

Conclusion

When NIR hyperspectral imaging was used to investigate oat samples with different hectolitre mass (HLM) values, PC6 score images clearly showed spectral differences between high and low HLM oat samples. Samples with similar HLM values produced spectra with fewer differences. PC1 and PC6 loading line plots (for all sample pairs) showed variation within PC6 explaining the difference between high and low HLM. These results were visualised with associated classification plots. Absorption peaks for water, protein and starch were prominent. It is most likely not protein or starch contents contributing to the variation between the samples but rather the compaction of the protein within the starch matrix. This can be related to density. The classification plots and images distinctly classified between kernels with low and high HLM (49.35 and 60.2 kg.hL^{-1}). The results found in this study shows promise for the future development to apply NIR hyperspectral imaging to classify between oat samples with different HLM values. Despite the use of mixed oat samples in this case, these preliminary results establish the possible use of hyperspectral imaging for the assessment of whole grain samples in breeding trials.

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Chapter 5

General discussion and conclusion

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General discussion and conclusion

International food grain markets trade based on grain quality. Hectolitre mass (HLM) is a measure of grain density and an important measurement used to characterise the quality of oat varieties. It is believed to be faster and easier to be carried out than a number of other measurements. The South African cereal industry relies on HLM as a grading factor. Two types of HLM equipment are currently being used in different grain producing and exporting countries. In the past, South Africa had been using a HLM device equipped with a funnel and a measuring cup of known volume (1 L). Currently, the grading regulations, with reference to HLM measurements of wheat in South Africa (SA), have been revised. HLM of wheat can now be determined with any suitable device that is compliant to the ISO 7971-3 standard (e.g. the device used in Germany for wheat). The United States of America (USA) and Canada use HLM devices with packing methods similar to that of the original SA device. The second type of HLM equipment is referred to as a chondrometer which is a cylindrical device. Chondrometers are typically used in Australia, United Kingdom (UK), Germany and France. In recent years, studies have been done to assess the variance in the measurement of HLM of maize (Engelbrecht, 2007) and wheat (Manley *et al.*, 2009). This current study was carried out to assess HLM devices from different countries (Australia, Canada, France, Germany, SA, UK and USA) and HLM measurements of locally produced South African oats. Samples were selected as such to represent a range of HLM values. In addition near infrared (NIR) hyperspectral imaging was used to attempt classification of oat samples with different HLM values.

Repeatable HLM values were obtained when results were compared within devices. Results obtained in this study conclusively showed differences to those results obtained when HLM devices were compared using maize (Engelbrecht, 2007) and wheat (Manley *et al.*, 2009). In the current study, the device used in Germany resulted in average HLM values significantly higher ($P<0.05$) compared to the other devices. In the previous studies, the Australian device was reported to have measured significantly higher ($P<0.05$) than the other devices when wheat and maize were used, respectively. A higher overall intra-class correlation (ICC) agreement value of 0.88 was found in the present study compared to a ICC agreement value of 0.52 reported in both studies of wheat (Manley et al., 2009) and maize (Engelbrecht, 2007), respectively. A higher overall ICC consistency value of 0.99 existed between respective devices in the current study. This value agrees with those reported earlier, i.e. ICC value of 0.94 for wheat (Manley *et al.*, 2009) and 0.99 for maize (Engelbrecht, 2007). It had been established conclusively that results obtained with the German, Canadian, Australian and UK devices were in close agreement when oats was used. Thus as was decided for wheat the German device should also replace the SA HLM device when grading oats

The average HLM values obtained from the respective devices when using oats were much lower compared to those values reported when HLM devices were compared using wheat and maize. One possible explanation for this would be the differences in density and shape of these cereal grains. Oats for example are less dense and one would expect oat samples to have lower HLM values than wheat which is denser. It had been also established in a study conducted in the Western Cape, South Africa that wheat cultivars had the highest density, with HLM values as high as 77.7 kg.hL^{-1}, while oat cultivars had the lowest density with values ranging from 41.0 kg.hL^{-1} (Brand *et al.*, 2003).

HLM values were found to increase by at least 1.7 kg.hL^{-1} when oat samples were rubbed before HLM measurements were performed. This observation is in agreement with results from previous studies (Cutler, 1940; Doehlert *et al.*, 2006). The effect of oat rubbing on HLM determination is to be attributed to the changes oat kernels undergo when rubbed. Polishing or mechanical rubbing shortens oat hulls and this is associated with a higher packing efficiency and thus higher HLM. The practice of rubbing oats before HLM measurements are performed is thus advisable.

Results presented in this and other studies (Greenway *et al.*, 1971; ISO, 1986; Manley *et al.*, 2009) indicated that an operator has a major influence on HLM determinations. Operator effect on oats HLM determination was found to exist when measurements were performed by operators with three different levels of skill. The unskilled operator measured HLM values significantly higher than those measured by the skilled and semi-skilled operators. This confirmed that training of operators is essential inspite of the HLM determination being a simple measurement to be performed. Even though, HLM measurements are easy to perform, it must be done with a high degree of consistency.

There was no apparent relationship between protein content and HLM of oats. Similar results were also observed by other investigators where poor correlation existed between protein content and HLM of wheat (Dexter *et al.*, 1987; Gaines, 1991; Preston *et al.*, 1995). Oat samples with higher HLM values would thus not necessarily have high protein content.

A relationship was found to exist between moisture content and the HLM of oat samples. Average HLM values of oat grains varied from 54.22 to 48.39 kg.hL^{-1}, indicating a decrease in HLM as the moisture was increased from *ca.* 10 to 18% moisture content. This significant decrease in HLM values with increased moisture content had been as well observed in maize (Engelbrecht, 2007; Rankin, 2009), wheat (Pushman, 1975; Gaines *et al.*, 1997; Lloyd *et al.*, 1999; Manley *et al.*, 2009) and faba bean (*Vicia faba L.*) (Altuntas & Yıldız, 2007). In the present study it was, however, found that when oat samples were conditioned back to their original moisture contents after drying, their HLM value decreased slightly below the HLM value obtained at its initial moisture content. The decrease in HLM value would be attributed to the swelling of the kernels with increased moisture content and roughing of the kernel surface. Packing efficiency would be expected to decrease with a resultant decrease in HLM values. It is thus worthy to note that an agreement must be reached in the oat industry on standard moisture contents when oats are traded.

Scanning electron microscopy (SEM) showed that high moisture content significantly affected oats microstructure due to the starch granules being affected. Conditioning to a high moisture content significantly increased the size of the oat starch granules. On the contrary, when oat grains were dried, the starch granules shrinked, but swelled again upon conditioning. This observation supports similar findings by Alsberg (1938). Starch is the major component of oats and the effect of increase in starch granules size contributes to the overall decrease in hectolitre mass (HLM) with increasing moisture content.

Near infrared (NIR) hyperspectral imaging results showed differences between two samples with low and high HLM values (difference of 10.85 kg.hL^{-1}). When the HLM difference between two samples were smaller (2.0 kg.hL^{-1}) fewer NIR spectroscopic differences were observed. The variation in NIR spectral data explaining the difference between the samples were linked to protein and starch. Despite the use of mixed oat samples these preliminary results establish the possible use of NIR hyperspectral imaging in evaluating oat samples differing in HLM.

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Appendices

Appendix 1

DEPARTMENT OF AGRICULTURE

DRAFT

AGRICULTURAL PRODUCT STANDARDS ACT, 1990

(ACT No. 119 OF 1990)

REGULATIONS RELATING TO THE GRADING, PACKING AND MARKING OF OATS INTENDED FOR SALE IN THE REPUBLIC OF SOUTH AFRICA

SCHEDULE

Definitions

1. Unless the context otherwise indicates, any word or expression in these regulations to which a meaning has been assigned in the Act shall have that meaning, and;

“animal filth” dead rodents, dead birds and dung;

“bag” means a bag manufactured from - -

(a) jute or phormium or a mixture of jute and phormium; or

(b) polypropilene that complies with SABS specification CKS632;

“bulk container” means any vehicle or container in which bulk oats is stored or transported;

“black, grey or brown oats” means kernels of pieces of kernels of oats covered by glumes

and is naturally black, grey or brown in colour;

"**consignment**" means

(a) a quantity of oats of the same class, which belongs to the same owner, delivered at any one time under cover of the same consignment note, delivery note or receipt note, or delivered by the same vehicle or bulk container, or loaded from the same bin of a grain elevator or from a ship's hold; or

(b) in the case where a quantity referred to in paragraph (a), is subdivided into different grades, each such quantity of each of the different grades;

“container” means a bag or bulk container;

"**cultivar list**" means the list of cultivars determined from time to time by the Executive Officer: Agricultural Product Standards and which is obtainable from the Executive Officer: Agricultural Product Standards, Private Bag X258, Pretoria, 0001;

"**damaged oats**" means oats --

(a) which have been damaged by insects;

(b) which have been distinctly discoloured (brown, dark brown or black) by external heat or as a result of heating caused by internal fermentation in oats with an excessive moisture content, excluding oat kernels in respect of which the discolouration is confined to the germ end;

(c) in which germination has proceeded to such an extent that the glume (lemma) covering the embryo has been broken or the developing rootlets and/or shoots are clearly visible; and

(d) which are immature and have a distinctly green colour;

“dehulled oats” means oats of which the enclosing glumes have been removed;

"**ergot sclerotia**" means the sclerotia of the fungus *Claviceps purpurea*; and "**ergot**" has a corresponding meaning;

"**field fungi infected oats**" means oats of which the kernels are visibly infected with fungi and has grey discolorations on any part of the kernel;

"**foreign matter**" means all material excluding oats, other grain and unthreshed ears and black, grey, brown and wild oats;

"**hectolitre mass**" means the mass in kilogram per hectolitre;

"**insect**" in relation to oats, means any live insect that is injurious to stored grain irrespective of the stage of development of that insect;

“oats” means kernels or pieces of kernels, with or without glumes, of the species *Avena* *sativa or Avena byzantia,* with the exception of oats that is naturally black, grey or brown in colour and wild oats;

"**other grain**" means the kernels or pieces of kernels of barley, triticale, maize, rye, sorghum and wheat;

"**poisonous seeds**" means the seeds or bits of seeds of plant species that may in terms of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972) represent a hazard to human or animal health when consumed, including seeds of *Argemone mexicana, Convolvulus spp.*, *Crotalaria spp., Datura spp., Ipomoea purpurea, Lolium temulentum, Ricinus communis* or *Xanthium spp.*;

"**screenings**" means all material that passes through the standard sieve;

“standard sieve” is a slotted sieve - -

(a) with a flat bottom of stainless steel metal sheet of 1,0 mm thickness with apertures 25 mm long and 1,5 mm wide with rounded ends. The spacing between the slots in the same row must be 2,43 mm wide and the spacing between the rows of slots must be 2,0 mm wide. The slots must be alternately orientated with a slot always opposite the solid inter segment of the next row of slots.

(b) of which the upper surface of the sieve is smooth;

(c) with a round frame of suitable material with an inner diameter of between 300 mm and 310 mm maximum and at least 50 mm high;

(d) that fits onto a tray with a solid bottom and must be at least 20 mm above the bottom of the tray;

"**storage fungi infected oats**" means oats kernels that are visibly infected with fungi, and that show white, blue, green, blackish or yellow fungal growth anywhere on the kernel;

"**the Act**" means the Agricultural Product Standards Act, 1990 (Act No. 119 of 1990);

"**unthreshed ears**" means ears and bits of ears of wheat, barley, triticale and rye that still contain seeds that are completely covered with glumes; and oats in which the first and second kernels are still attached;

"wild oats" means kernels and pieces of kernels of the species *Avena* excluding *Avena sativa, A. nuda* and *A. byzantina*;

Restrictions on sale of oats

2. (1) No person shall sell a consignment of wheat in the Republic of South Africa -

   (a) unless the oats is sold according to the classes set out in regulation 3;

   (b) unless the oats complies with the standards for the classes set out in regulation 4;

   (c) unless the oats, where applicable, complies with the grades of oats and the standards for grades set out in regulations 5 and 6 respectively;

   (d) unless the oats is packed in accordance with the packing requirements set out in regulation 7;

   (e) unless the containers or sale documents, as the case may be, are marked in accordance with the marking requirements set out in regulation 8; and

   (f) if such oats contains a substance that renders it unfit for human consumption or for processing into or utilisation thereof as food or feed.

(2) The Executive Officer may grant written exemption, entirely or partially, to any person on such conditions as he or she may deem necessary, from the provisions of subregulation (1).

PART I

QUALITY STANDARDS

Classes of oats

3. The classes of oats are --

   (a) Class Oats; and

   (b) Class Other Oats.

Standards for classes

4. (1) Notwithstanding the provisions of sub regulations (2) and (3), all consignments of oats must --

   (a) be free from any toxin, chemical or other substances that renders it unsuitable for human consumption or for processing into or utilisation thereof as food or feed and may not exceed the permissible deviations regarding aflatoxin in terms of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972);

   (b) contain not more poisonous seeds or ergot sclerotia than permitted in terms of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972);

   (c) be free from organisms of phytosanitary importance as determined in terms of the Agricultural Pest Act, 1983 (Act No. 36 of 1983);

   (d) be free from mould infected, sour and rancid other grain, foreign matter and any other matter;

   (e) be free from any undesired odour, taste or colour not typical of undamaged and sound oats;

   (f) be free from animal filth;

   (g) may not exceed the maximum residue levels prescribed for agricultural remedies, according to the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies, 1947 (Act no 36 of 1947) permissible for the control of pests and deceases on oats;

(h) be free from fire damaged oats;

(i) with the exception of Class Other Oats, be free from insects;

(j) with the exception of Class Other Oats, be free from oats and other grain kernels smeared by smut and may not contain more than four smut masses per 100 g oats; and

(k) with the exception of Class Other Oats, have a moisture content not exceeding 12.5 per cent.

(2) A consignment of oats shall be classified as Class Other Oats if it does not comply with the standards for Class Oats.

Grades of oats

5. (1) The grades for Class Oats shall be as follows:

(a) Grade 1;

(b) Grade 2; and

(c) Grade 3;

(2) No grades are determined for Class Other Oats.

Standards for grades of oats

6. (1) Subject to the provisions of sub regulation (2), a consignment of oats shall be graded as

(a) Grade 1 if the nature of deviation, specified in column 1 of Table 1 of the Annexure, in that consignment does not exceed the percentage specified in column 2 of the said table opposite the deviation concerned;

(b) Grade 2 if the nature of deviation, specified in column 1 of Table 1 of the Annexure, in that consignment does not exceed the percentage specified in column 3 of the said table opposite the deviation concerned;

(c) Grade 3 if the nature of deviation, specified in column 1 of Table 1 of the Annexure, in that consignment does not exceed the percentage specified in column 4 of the said table opposite the deviation concerned;

(2) (a) The minimum hector litre masses for the different grades are as follows:

(i) Grade 1 – 50 kg;

(ii) Grade 2 – 46 kg; and

(iii) Grade 3 – 38 kg;

PART II

PACKING AND MARKING REQUIREMENTS

Packing requirements

7. Oats of different grades shall be packed in different containers, or stored separately.

Marking requirements

8. (1) Every container or the accompanying sale documents of a consignment of oats shall be marked or endorsed by means of appropriate symbols specified in subregulation (2), with

(a) the class of the oats; and

(b) the grade.

(2) The symbols referred to in subregulation (1) shall appear in the order of class and grade.

(3) The symbols used to indicate the different --

(a) classes shall be --

(i) H in the case of Class Oats; and

(ii) O in the case of Class Other Oats;

(b) grades shall be

(i) 1 in the case of Grade 1;

(ii) 2 in the case of Grade 2; and

(iii) 3 in case of Grade 3;

PART III

SAMPLING

Taking of sample

9. (1) A sample of a consignment of oats shall --

(a) in the case of oats delivered in bags and subject to regulation 10, be obtained by sampling at least ten per cent of the bags, chosen from that consignment at random, with a bag probe: Provided that at least 25 bags in a consignment shall be sampled and where a consignment consists of less than 25 bags, all the bags in that consignment shall be sampled; and

(b) in the case of oats delivered in bulk and subject to regulation 10, be obtained by sampling that consignment throughout the whole depth of the layer, in at least six different places, chosen at random in that bulk quantity, with a bulk sampling apparatus.

(2) The collective sample obtained in subregulation (1)(a) or (b) shall --

(a) have a total mass of at least 5 kg; and

(b) be thoroughly mixed by means of dividing before further examination.

(3) If it is suspected that the sample referred to in sub regulation (1)(a) is not

representative of that consignment, an additional five per cent of the remaining bags, chosen from that consignment at random, shall be emptied into a suitable bulk container and sampled in the manner contemplated in subregulation (1)(b).

(4) If it is suspected that the sample referred to in sub regulation (1)(b) is not

representative of that consignment, an additional representative sample shall be obtained by using an alternative sampling pattern, apparatus or method.

(5) A sample taken in terms of these regulations shall be deemed to be representative of the consignment from which it was taken.

Sampling if contents differ

10. (1) If, after an examination of the oats taken from different bags in a consignment

in terms of regulation 9(1)(a), it appears that the contents of those bags differ substantially --

(a) the bags concerned shall be placed separately;

(b) all the bags in the consignment concerned shall be sampled with a bag probe in order to do such separation; and

(c) each group of bags with similar contents in that consignment shall for the purposes of these regulations be deemed to be a separate consignment.

(2) If, after the discharge of a consignment of oats in bulk has commenced, it is

suspected that the consignment could be of a class or grade other than that determined by means of the initial sampling, the discharge shall immediately be stopped and the part of the consignment remaining in the bulk container as well as the oats already in the hopper shall be sampled anew with a bulk sampling apparatus or by catching at least 20 samples, by means of a suitable container, at regular intervals throughout the whole offloading period from the stream of oats flowing in bulk.

Working sample

11. A working sample is obtained by dividing the representative sample of the consign

ment according to the ICC (International Association for Cereal Chemistry) 101/1 method.

PART IV

DETERMINATION OF OTHER SUBSTANCES

Determination of undesirable odours and harmful substances

12. A consignment of oats or a sample of a consignment of oats shall be sensorial assessed or chemically analysed in order to determine --

    (a) whether it contains a substance that renders the oats unfit for human consumption or for processing into or for utilisation as food or feed; and

    (c) whether it has a musty, sour, rancid or other undesirable odour: Provided that a working sample of unscreened oats that is ground in a grain mill to a fine meal may be used for the determination concerned.

PART V

DETERMINATION OF HECTOLITRE MASS AND MOISTURE CONTENT

Determination of the hectolitre mass

13. The hectolitre mass of a consignment of oats may be determined by any suitable instrument: Provided that the instrument comply with the specifications detailed in ISO 7971-3.

Determination of moisture content

14. The moisture content of a consignment oats may be determined by any suitable method: Provided that the results thus obtained is in accordance with the maximum permissible deviation for a class 1 moisture meter as detailed in ISO 7700/1 based on the results of the 72 hour, 103°C oven dried method [AACC (American Association for Cereal Chemistry) Method 44-15A].

PART VI

DETERMINATION OF PERCENTAGE DEVIATIONS

Determination of the percentage black, grey or brown oats and wild oats

15. The percentage black, gray or brown oats and wild oats in a consignment of oats shall be determined as follows:

    (a) Obtain a working sample of at least 50 g of a screened un-rubbed sample.

    (b) Remove all black, grey or brown oats and wild oats by hand and determine the mass thereof.
    (c) Express the mass thus determined as a percentage of the working sample.
    (d) Such percentage represents the percentage black, grey or brown oats and wild oats in the consignment.

Determination of the percentage dehulled oats

16. The percentage dehulled oats in a consignment of oats shall be determined as follows:
    (e) Obtain a working sample of at least 25 g of a screened un-rubbed sample.
    (f) Remove all dehulled oats by hand and determine the mass thereof.
    (g) Express the mass thus determined as a percentage of the working sample.
    (h) Such percentage represents the percentage dehulled oats in the consignment.

Determination of percentage screenings

17. The percentage screenings in a consignment of oats shall be determined as follows:
    (a) Obtain a working sample of at least 400 g.
    (b) Place the sample on the standard sieve and screen the sample by moving the sieve 50 strokes to and fro, alternately away from and towards the operator of the sieve, in the same direction as the long axes of the slots of the sieve. Move the sieve, which rests on a table

or other suitable smooth surface, 250 mm to 460 mm away from and towards the operator with each stroke. The prescribed 50 strokes must be completed within 50 to 60 seconds: Provided that the screening process may also be performed in some or other container or an automatic sieving apparatus.

(c) Determine the mass of the material that has passed through the sieve and express it as a percentage of the mass of the working sample.

(d) Such percentage represents the percentage screenings in the consignment.

Determination of the percentage foreign matter

18. The percentage foreign matter in a consignment of oats shall be determined as follows:

(a) Obtain a working sample of at least 50 g of a screened un-rubbed sample.

(b) Remove all foreign matter by hand and determine the mass thereof.

(c) Express the mass thus determined as a percentage of the working sample.

(d) Such percentage represents the percentage foreign matter in the consignment.

Determination of the percentage sand, gravel and stones

19. The percentage sand, gravel and stones in a consignment of oats shall be determined as follows:

(a) Obtain a working sample of at least 100 g of a screened un-rubbed sample.

(b) Remove all foreign matter by hand and determine the mass thereof.

(c) Express the mass thus determined as a percentage of the working sample.

(d) Such percentage represents the percentage sand, gravel and stones in the consignment.

Determination of the percentage damaged oats

20. The percentage damaged oats in a consignment of oats shall be determined as follows:

(a) Obtain a working sample of at least 25 g of a screened un-rubbed sample.
(b) Remove all damaged oats by hand and determine the mass thereof.
(c) Express the mass thus determined as a percentage of the working sample.
(d) Such percentage represents the percentage damaged oats in the consignment.

Determination of the percentage heat damaged oats

21. The percentage heat damaged oats in a consignment of oats shall be determined as follows:
    (a) Obtain a working sample of at least 100 g of a screened un-rubbed sample.
    (b) Remove all heat damaged oats by hand and determine the mass thereof.
    (c) Express the mass thus determined as a percentage of the working sample.
    (d) Such percentage represents the percentage heat damaged oats in the consignment.

Determination of the percentage other grain and un-threshed ears

22. The percentage other grain and un-threshed ears in a consignment of oats shall be determined as follows:
    (a) Obtain a working sample of at least 50 g of a screened un-rubbed sample.
    (b) Remove all other grain and un-threshed ears by hand and determine the mass thereof.
    (c) Express the mass thus determined as a percentage of the working sample.
    (d) Such percentage represents the percentage other grain and un-threshed ears in the consignment.

Determination of the percentage storage fungi infected oats

23. The percentage storage infected oats in a consignment of oats shall be determined as follows:
    (a) Obtain a working sample of at least 25 g of a screened un-rubbed sample.
    (b) Remove all storage fungi infected oats by hand and determine the mass thereof.
    (c) Express the mass thus determined as a percentage of the working sample.
    (d) Such percentage represents the percentage storage fungi infected oats in the consignment.

Determination of the percentage field fungi infected oats

24. The percentage storage infected oats in a consignment of oats shall be determined as follows:
    (a) Obtain a working sample of at least 25 g of a screened un-rubbed sample.
    (b) Remove all field fungi infected oats by hand and determine the mass thereof.
    (c) Express the mass thus determined as a percentage of the working sample.
    (d) Such percentage represents the percentage field fungi infected oats in the consignment.

PART VII

Offence and penalties

25. Any person who contravenes or fails to comply with any provision of these regulations shall be guilty of an offence and upon conviction be liable to a fine of not exceeding R8 000 or to imprisonment for a period not exceeding two years, or to both that fine or imprisonment.

ANNEXURE/AANHANGSEL

TABLE 1/TABEL 1

STANDARDS FOR GRADES OF CLASS OATS/

STANDAARDE VIR GRADE VAN KLAS HAWER

Nature of deviation/ *Aard van afwyking*	**Maximum percentage permissible deviation (m/m)/ Maksimum persentasie toelaatbare afwyking (m/m)**		
	Grade 1/ Graad 1	**Grade 2/ Graad 2**	**Feed grade/ Voergraad**
1	**2**	**3**	**4**
(a) Black, gray or brown oats or wild oats/Swart, grys of bruin hawer en wilde hawer [Reg. 15]	1	2	4
(b) Storage fungi infected oats /Opberging swambesmette hawer [Reg. 23]	0,5	0,5	0,5

Nature of deviation/ ***Aard van afwyking***	**Maximum percentage permissible deviation (m/m)/** **Maksimum persentasie toelaatbare afwyking (m/m)**		
	Grade 1/ **Graad 1**	**Grade 2/** **Graad 2**	**Feed grade/** **Voergraad**
1	**2**	**3**	**4**
(c) Field fungi infected oats/ Land swambesmette hawer [Reg. 24]	3	3	6
(d) Dehulled oats/Uitgedopte hawer [Reg. [16]	2	5	*
(e) Screenings/Sifsels [Reg. [17]	2	2	20
(f) Sand, gravel and stones/Sand, gruis en klippies [Reg. 18]	0,5	0,5	0,5

Nature of deviation/ ***Aard van afwyking***	**Maximum percentage permissible deviation (m/m)/** **Maksimum persentasie toelaatbare afwyking (m/m)**		
	Grade 1/ **Graad 1**	**Grade 2/** **Graad 2**	**Feed grade/** **Voergraad**
1	**2**	**3**	**4**
(g) Foreign matter including sand, gravel and stones: Provided that such deviations are individually within the limits specified in item (f)/ Vreemde voorwerpe met inbegrip van sand, gruis en klippies: Met dien verstande dat sodanige afwykings individueel binne die perke is in item (f) aangegee [Reg. 18]	1	1	10
(h) Heat-damaged kernels/Hittebeskadigde korrels [Reg. 21]	0,5	0,5	20

Nature of deviation/ Aard van afwyking	**Maximum percentage permissible deviation (m/m)/ Maksimum persentasie toelaatbare afwyking (m/m)**		
	Grade 1/ Graad 1	**Grade 2/ Graad 2**	**Feed grade/ Voergraad**
1	**2**	**3**	**4**
(i) Damaged kernels, including heat-damaged kernels: Provided that such deviations are individually within the limit specified in item (h) /Beskadigde korrels met inbegrip van hittebeskadigde korrels: Met dien verstande dat sodanige afwyking individueel binne die perke is in item (h) aangegee [Reg. 20]	3	3	20
(j) Other grain and unthreshed ears/ Ander graan en ongedorste are [Reg. 22]	2	2	15

Nature of deviation/ ***Aard van afwyking***	**Maximum percentage permissible deviation (m/m)/** **Maksimum persentasie toelaatbare afwyking (m/m)**		
	Grade 1/ **Graad 1**	**Grade 2/** **Graad 2**	**Feed grade/** **Voergraad**
1	**2**	**3**	**4**
(j) Deviations in items (g), (i) and (j) collectively: Provided that such deviations are individually within the limits of the said items/ Afwykings in items (g), (i) en (j) gesamentlik: Met dien verstande dat sodanige afwykings individueel binne die perke van genoemde items is	3	3	20

Appendix 2

Table 1 Gram to hectolitre mass conversion chart for oats of the Kern 220/222 Grain Sampler

g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}
355	35.5	394	39.3	433	43.25	472	47.2	511	51.15
356	35.45	395	39.4	434	43.35	473	47.3	512	51.25
357	35.55	396	39.5	435	43.45	474	47.4	513	51.35
358	35.65	397	39.6	436	43.55	475	47.5	514	51.45
359	35.75	398	39.7	437	43.65	476	47.6	515	51.6
360	35.85	399	39.8	438	43.75	477	47.7	516	51.7
361	35.95	400	39.9	439	43.85	478	47.85	517	51.8
362	36.05	401	40.0	440	44.0	479	47.95	518	51.9
363	36.15	402	40.1	441	44.1	480	48.05	419	52.0
364	36.25	403	40.25	442	44.2	481	48.15	520	52.1
365	36.4	404	40.35	443	44.3	482	48.25	521	52.2
366	36.5	405	40.45	444	44.4	483	48.35	522	52.3
367	36.6	406	40.55	445	44.5	484	48.45	523	52.4
368	36.7	407	40.65	446	44.6	485	48.55	524	52.5
369	36.8	408	40.75	447	44.7	486	48.65	525	52.6
370	36.9	409	40.85	448	44.8	487	48.75	526	52.7
371	37.0	410	40.95	449	44.9	488	48.85	527	52.8
372	37.1	411	41.05	450	45.0	489	48.95	528	52.9
373	37.2	412	41.15	451	45.1	490	49.05	529	53.0
374	37.3	413	41.25	452	45.2	491	49.15	530	53.1
375	37.4	414	41.35	453	45.3	492	49.25	531	53.2
376	37.5	415	41.45	454	45.4	493	49.35	532	53.3
377	37.6	416	41.55	455	45.5	494	49.45	533	53.4
378	37.7	417	41.65	456	45.6	495	49.55	534	53.5
379	37.8	418	41.75	457	45.7	496	49.65	535	53.6
380	37.9	419	41.85	458	45.8	497	49.75	536	53.7
381	38.0	420	41.95	459	45.9	498	59.85	537	53.8
382	38.1	421	42.05	460	46.0	499	49.95	538	53.9
383	38.2	422	42.15	461	46.1	500	50.05	539	54.0
384	38.3	423	42.25	462	46.2	501	50.15	540	54.1
385	38.4	424	42.35	463	46.3	502	50.25	541	54.2
386	38.5	425	42.45	464	46.4	503	50.35	542	54.3
387	38.6	426	42.55	465	46.5	504	50.45	543	54.4
388	38.7	427	42.65	466	46.6	505	50.55	544	54.5
389	38.8	428	42.75	467	46.7	506	50.65	545	54.6
390	38.9	429	42.85	468	46.8	507	50.75	546	54.7
391	39.0	430	42.95	469	46.9	508	50.85	547	54.8
392	39.1	431	43.05	470	47.0	509	50.95	548	54.9
393	39.2	432	43.15	471	47.1	510	51.05	549	55.0

Table 1 continued

g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}	g.L^{-1}	kg.hL^{-1}
550	55.1	589	59.05	628	63.05
551	55.2	590	59.2	629	63.15
552	55.3	591	59.3	630	63.25
553	55.45	592	59.4	631	63.35
554	55.55	593	59.5	632	63.45
555	55.65	594	59.6	633	63.55
556	55.75	595	59.7	634	63.65
557	55.85	596	59.8	635	63.75
558	55.95	597	59.9	636	63.85
559	56.05	598	60.0	637	63.95
560	56.15	599	60.1	638	64.05
561	56.25	600	60.2	639	64.15
562	56.35	601	60.3	640	64.25
563	56.45	602	60.4	441	64.35
564	56.55	603	60.5	642	64.45
565	56.65	604	60.6	643	64.55
566	56.75	605	60.7	644	64.65
567	56.85	606	60.8	645	64.75
568	56.95	607	60.9	646	64.85
569	57.05	608	61.0	647	64.95
570	57.15	609	61.1	648	65.05
571	57.25	610	61.2	649	65.15
572	57.35	611	61.3	650	65.25
573	57.45	612	61.4	651	65.35
574	57.55	613	61.5	652	65.45
575	57.65	614	61.6	653	65.55
576	57.75	615	61.7	654	65.65
577	57.85	616	61.8	655	65.75
578	57.95	617	61.9	656	65.85
579	58.05	618	62.0	657	65.95
580	58.15	619	62.1	658	66.05
581	58.25	620	62.2	659	66.15
582	58.35	621	62.3	-	-
583	58.45	622	62.4	-	-
584	58.55	623	62.5	-	-
585	58.65	624	62.6	-	-
586	58.75	625	62.7	-	-
587	58.85	626	62.8	-	-
588	58.95	627	62.9	-	-

Table 2 Gram to hectolitre mass conversion chart for oats of the Ohaus 500 mL measure and Cox funnel

g.0.5 L^{-1}	kg.hL^{-1}	g.0.5 L^{-1}	kg.hL^{-1}	g.0.5 L^{-1}	kg.hL^{-1}	g.0.5 L^{-1}	kg.hL^{-1}
179	39.9	218	47.6	257	55.4	296	63.1
180	40.1	219	47.8	258	55.6	297	63.3
181	40.3	220	48	259	55.7	298	63.5
182	40.5	221	48.2	260	55.9	299	63.7
183	40.7	222	48.4	261	56.1	300	63.9
184	40.9	223	48.6	262	56.3	-	-
185	41.1	224	48.8	263	56.5	-	-
186	41.3	225	49	264	56.7	-	-
187	41.5	226	49.2	265	56.9	-	-
188	41.6	227	49.4	266	57.1	-	-
189	41.8	228	49.6	267	57.3	-	-
190	42	229	49.8	268	57.5	-	-
191	42.2	230	50	269	57.7	-	-
192	42.4	231	50.2	270	57.9	-	-
193	42.6	232	50.4	271	58.1	-	-
194	42.8	233	50.6	272	58.3	-	-
195	43	234	50.8	273	58.5	-	-
196	43.2	235	51.0	274	58.7	-	-
197	43.4	236	51.2	275	58.9	-	-
198	43.6	237	51.4	276	59.1	-	-
199	43.8	238	51.6	277	59.3	-	-
200	44.0	239	51.8	278	59.5	-	-
201	44.2	240	52.0	279	59.7	-	-
202	44.4	241	52.2	280	59.9	-	-
203	44.6	242	52.4	281	60.1	-	-
204	44.8	243	52.6	282	60.3	-	-
205	45.0	244	52.8	283	60.5	-	-
206	45.2	245	53	284	60.7	-	-
207	45.4	246	53.2	285	60.9	-	-
208	45.6	247	53.4	286	61.1	-	-
209	45.8	248	53.6	287	61.3	-	-
210	46	249	53.8	288	61.5	-	-
211	46.2	250	54.0	289	61.7	-	-
212	46.4	251	54.2	290	61.9	-	-
213	46.6	252	54.4	291	62.1	-	-
214	46.8	253	54.6	292	62.3	-	-
215	47	254	54.8	293	62.5	-	-
216	47.2	255	55.0	294	62.7	-	-
217	47.4	256	55.2	295	62.9	-	-

Table 3 Gram to hectolitre mass conversion chart of the Easi-Way Portable Test Weight Kit

Gram	**kg.hL^{-1}**
346	70.0
348	70.4
350	70.8
352	71.2
354	71.6
356	72.0
358	72.4
360	72.4
362	73.2
364	73.6
366	74.0
368	74.4
370	74.8
372	75.2
374	75.6
376	76.0
378	76.4
380	76.8
382	77.2
384	77.6
386	78.0
388	78.4
390	78.8
392	79.2
394	79.6
396	80.0
398	80.4
400	80.8
402	81.2
404	81.6
406	82.0
408	82.4
410	82.8
412	83.2
414	83.6
416	84.0
418	84.4
420	84.8
422	85.2

Table 4 Gram to test weight (lb.bu^{-1}) conversion chart of the Seedburo 151 Filling Hopper with a quart cup

Gram	lb.bu^{-1}	Gram	lb.bu^{-1}	Gram	lb.bu^{-1}	Gram	lb.bu^{-1}	Gram	lb.bu^{-1}
758	53.5	778	54.9	798	56.3	818	57.5	838	59.1
758.5	53.5	778.5	54.9	798.5	56.3	818.5	57.5	838.5	59.2
759	53.5	779	55.0	799	56.4	819	57.8	839	59.2
759.5	53.6	779.5	55.0	799.5	56.4	819.5	57.8	839.5	59.2
760	53.6	780	55.0	800	56.4	820	57.8	840	59.3
760.5	53.7	780.5	55.1	800.5	56.5	820.5	57.9	840.5	59.3
761	53.7	781	55.1	801	56.5	821	57.9	841	59.3
761.5	53.7	781.5	55.1	801.5	56.5	821.5	58.0	841.5	59.4
762	53.8	782	55.2	802	56.6	822	58.0	842	59.4
762.5	53.8	782.5	55.2	802.5	56.6	822.5	58.0	842.5	59.4
763	53.8	783	55.2	803	56.6	823	58.1	843	59.5
763.5	53.9	783.5	55.3	803.5	56.7	825.5	58.1	843.5	59.5
764	53.9	784	55.3	804	56.7	824	58.1	844	59.5
764.5	53.9	784.5	55.3	804.5	56.8	824.5	58.2	844.5	59.6
765	54.0	785	55.4	805	56.8	825	58.2	845	59.6
765.5	54.0	785.5	55.4	805.5	56.8	825.5	58.2	845.5	59.6
766	54.0	786	55.5	806	56.9	826	58.3	846	59.7
766.5	54.1	786.5	55.5	806.5	56.9	826.5	58.3	846.5	59.7
767	54.1	787	55.5	807	56.9	827	58.3	847	59.8
767.5	54.1	787.5	55.6	807.5	75.0	827.5	58.4	847.5	59.8
768	54.2	788	55.6	808	75.0	828	58.4	848	59.8
768.5	54.2	788.5	55.6	808.5	75.0	828.5	58.4	848.5	59.9
769	54.3	789	55.7	809	57.1	829	58.5	849	59.9
769.5	54.3	789.5	55.7	809.5	57.1	829.5	58.5	849.5	59.9
770	54.3	790	55.7	810	57.1	830	58.6	850	60.0
770.5	54.4	790.5	55.8	810.5	57.2	830.5	58.6	850.5	60.0
771	54.4	791	55.8	811	57.2	831	58.6	851	60.0
771.5	54.4	791.5	55.8	811.5	57.2	831.5	58.7	851.5	60.1
772	54.5	792	55.9	812	57.3	832	58.7	852	60.1
772.5	54.5	792.5	55.9	812.5	57.3	832.5	58.7	852.5	60.1
773	54.5	793	55.9	813	57.4	833	58.8	853	60.2
773.5	54.6	793.5	56.0	813.5	57.4	833.5	58.8	853.5	60.2
774	54.6	794	56.0	814	57.4	834	58.8	854	60.2
774.5	54.6	794.5	56.1	814.5	57.5	834.5	58.9	854.5	60.3
775	54.7	795	56.1	815	57.5	835	58.9	855	60.3
775.5	54.7	795.5	56.1	815.5	57.5	835.5	58.9	855.5	60.4
776	54.7	796	56.2	816	57.6	836	59.0	856	60.4
776.5	54.8	796.5	56.2	816.5	57.6	836.5	59.0	856.5	60.4
777	54.8	797	56.2	817	57.6	837	59.0	857	60.5
777.5	54.8	797.5	56.3	817.5	57.7	837.5	59.1	857.5	60.5

Table 4 continued

Gram	lb.bu^{-1}	Gram	lb.bu^{-1}	Gram	lb.bu^{-1}
858	60.5	878	61.9	898	63.4
858.5	60.6	878.5	62.0	898.5	63.4
859	60.6	879	62.0	899	63.4
859.5	60.6	879.5	62.0	899.5	63.5
860	60.7	880	62.1	900	63.5
860.5	60.7	880.5	62.1	900.5	63.5
861	60.7	881	62.2	901	63.6
861.5	60.8	881.5	62.2	901.5	63.6
862	60.8	882	62.2	902	63.6
862.5	60.8	882.5	62.3	902.5	63.7
863	60.9	883	62.3	903	63.7
863.5	60.9	883.5	62.3	903.5	63.7
864	61	884	62.4	904	63.8
864.5	61	884.5	62.4	904.5	63.8
865	61	885	62.4	905	63.8
865.5	61.1	885.5	62.5	905.5	63.9
866	61.1	886	62.5	906	69.9
866.5	61.1	886.5	62.5	906.5	64.0
867	61.2	887	62.6	907	64.0
867.5	61.2	887.5	62.6	907.5	64.0
868	61.2	888	62.6	908	64.1
868.5	61.3	888.5	62.7	908.5	64.1
869	61.3	889	62.7	909	64.1
869.5	61.3	889.5	62.8	909.5	64.2
870	61.4	890	62.8	910	64.2
870.5	61.4	890.5	62.8	910.5	64.2
871	61.4	891	62.9	911	64.3
871.5	61.5	891.5	62.9	911.5	64.3
872	61.5	892	62.9	912	64.3
872.5	61.6	892.5	63.0	912.5	64.4
873	61.6	893	63.0	913	64.4
873.5	61.6	893.5	63.0	913.5	64.4
874	61.7	894	63.1	914	64.5
874.5	61.7	894.5	63.1	914.5	64.5
875	61.7	895	63.1	915	64.6
875.5	61.8	895.5	63.2	915.5	64.6
876	61.8	896	63.2	916	64.6
876.5	61.8	896.5	63.2	916.5	64.7
877	61.9	897	63.3	917	64.7
877.5	61.9	897.5	63.3	917.5	64.7

Test weight in pounds per bushel (lb.bu^{-1}) was converted to hectolitre mass (kg.hL^{-1}) using the following formula: lb.bu^{-1} × 1.287.

Appendix 3

Table 1 Hectolitre mass values for the 10 repetitions as performed on each device using one oat sample. Experiment 1: variation between HLM devices using sub-samples.

Germany	Australia	UK	Canada	France	SA	USA
53.60	53.01	53.37	53.80	52.57	52.15	51.20
53.10	53.32	53.92	53.40	52.56	52.59	51.07
52.60	52.96	52.54	53.60	52.87	51.37	50.68
53.20	53.21	53.02	53.40	53.42	51.06	51.42
53.5o	52.61	53.6	53.40	53.72	51.14	50.20
53.80	53.66	52.02	53.80	53.43	51.30	51.16
52.50	52.64	53.89	53.80	52.94	51.94	51.15
53.60	53.39	53.27	52.20	52.95	51.17	50.38
53.70	53.54	53.52	51.60	53.69	51.88	50.55
53.90	53.54	52.10	53.20	53.27	52.14	51.29

Table 2 Hectolitre mass values for 10 repetitions on each HLM device using single work samples of three oat samples. Experiment 2: variation in repeatability within and variation between the HLM devices using single work samples.

Sample	Germany	Australia	UK	Canada	France	SA	USA
1	54.9	54.26	54.02	54.4	53.69	51.8	51.01
1	54.7	54.11	54.67	54.6	53.65	51.67	51.15
1	54.9	55.03	54.72	54.4	53.56	51.65	51.36
1	55.3	54.31	54.06	55.2	53.8	51.69	51.15
1	54.8	54.56	54.77	54.8	53.75	51.54	51.67
1	54.9	54.44	54.48	54.6	53.78	51.41	50.95
1	54.7	54.61	54.49	54.4	53.81	52.02	51.15
1	54.4	54.53	54.6	54.8	53.7	52.35	50.97
1	54.8	54.31	54.31	55.4	53.76	52.27	51.23
1	55.2	54.83	53.87	54.6	53.76	51.75	50.89
2	57.85	57.69	57.61	57.9	57	55.45	55.07
2	57.85	57.78	57.49	57.5	57.2	55.91	55.23
2	57.95	57.92	57.57	57.9	57.12	56.23	54.96
2	57.95	57.99	56.98	57.9	57.09	55.64	54.96
2	58.25	57.54	57.74	57.5	57.27	55.56	55.49
2	57.95	57.38	57.47	57.7	57.24	56.05	55.1
2	58.25	57.39	57.48	58.3	57.31	56.41	55.24
2	57.95	57.76	57.63	57.5	57.08	55.89	54.98
2	58.25	57.02	57.92	57.9	57.23	55.57	55.15
2	58.25	57.1	57.87	58.3	57.2	55.99	55.03
3	55.85	55.49	55.47	55.2	55.02	53.73	52.25
3	55.75	56.13	55.18	55.7	55	53.79	52.57
3	55.85	55.79	55.54	55.4	55.31	53.53	52.55
3	56.15	55.37	55.45	56.1	55.31	53.58	52.55
3	55.65	55.15	55.24	55.9	55.39	53.86	52.43
3	55.85	55.73	55.08	55.9	55.07	53.3	52.33
3	55.65	55.5	55.7	55.6	55.24	53.67	52.48
3	55.95	55.84	55.09	55.6	55.26	53.44	52.66
3	55.75	55.88	55.62	56.3	55.03	53.89	52.36
3	55.75	55.53	55.38	55.9	55.16	54.11	52.54

Table 3 Intra-class correlation (ICC) agreement and (ICC) consistency between the HLM devices using the three oat samples. Experiment 2: Variation in repeatability within and variation between the HLM devices using single work samples.

HLM equipment		ICC agreement	ICC consistency
Germany	Australia	0.93	0.96
Germany	UK	0.92	0.97
Germany	Canada	0.97	0.97
Germany	France	0.82	0.98
Germany	SA	0.41	0.93
Germany	USA	0.29	0.97
Australia	UK	0.94	0.94
Australia	Canada	0.92	0.93
Australia	France	0.89	0.96
Australia	SA	0.47	0.92
Australia	USA	0.32	0.94
UK	Canada	0.93	0.95
UK	France	0.93	0.97
UK	SA	0.51	0.93
UK	USA	0.35	0.96
Canada	France	0.85	0.97
Canada	SA	0.44	0.94
Canada	USA	0.3	0.95
France	SA	0.65	0.97
France	USA	0.44	0.98
SA	USA	0.86	0.97
Overall		0.54	0.95

Table 4 Hectolitre mass values for the duplicate measurements on each sub-sample using single work samples of 10 oat samples. Experiment 3: comparison of the HLM devices using a single work samples of 10 oat samples.

Sample	Sub	Rep	Germany	Australia	UK	Canada	France	SA	USA
1	a	1	49.35	50.44	47.78	48.6	47.25	45.6	45.31
1	a	2	49.35	51.25	48.22	49.2	47.25	46.06	44.91
1	b	1	49.35	49.72	49.2	49	47.39	46.47	45.16
1	b	2	49.45	50.24	49.13	48.8	47.41	45.95	45.4
1	c	1	48.75	48.56	48.33	48.6	47.2	45.84	45.08
1	c	2	48.75	50.03	48.36	48.6	47.08	45.66	44.89
2	a	1	59.4	58.86	59.16	59.1	57.85	55.85	55.45
2	a	2	59.7	59.25	59.34	60.1	57.6	56.54	55.26
2	b	1	59.2	59.14	59.16	60.5	57.75	56.37	55.07
2	b	2	59.5	59.4	58.61	59.7	57.74	56.5	55.31
2	c	1	58.95	58.32	58.84	58.9	57.62	55.91	55.12
2	c	2	58.95	58.65	58.25	59.3	57.55	56.16	54.94
3	a	1	57.65	58.19	56.12	56.1	55.47	53.69	53.17
3	a	2	57.45	58.15	56.99	56.7	55.74	54.04	53.1
3	b	1	57.55	58.5	57.03	57.1	55.98	54.58	53.16
3	b	2	57.85	58.47	56.78	56.1	56.16	54.14	53.32
3	c	1	57.35	58.23	57.43	57.3	55.63	54	53.42
3	c	2	57.75	57.29	56.39	57.5	55.33	54.32	53.33
4	a	1	55.65	55.95	55.42	56.3	54.91	52.98	52.17
4	a	2	55.75	55.77	54.93	56.3	54.56	53.89	52.26
4	b	1	56.45	55.29	56.05	55.9	54.99	53.6	52.63
4	b	2	56.55	56.27	55.93	57.1	54.63	53.17	52.66
4	c	1	56.05	56.6	55.84	56.3	54.64	53.28	52.38
4	c	2	56.25	56.97	55.36	56.1	54.87	53.51	52.52
5	a	1	50.75	51.06	49.92	52.2	48.96	48	47.11
5	a	2	50.75	50.12	50.33	50.8	49.25	48.53	47.02
5	b	1	50.95	50.05	51.21	51.2	49.82	48.82	47.35
5	b	2	51.25	50.87	50.89	52	49.75	48.58	47.61
5	c	1	50.95	50.76	49.92	50.4	49.48	48.71	47.49
5	c	2	50.55	49.92	49.76	52	49.67	48.12	47.26
6	a	1	58.45	58.04	57.28	58.5	57.35	56.56	55.06
6	a	2	59.05	58.44	57.31	59.3	57.79	56.07	55.48
6	b	1	58.85	58.34	57.81	58.3	57.29	56.14	54.53
6	b	2	58.85	58.27	57.69	57.7	57.37	56.69	54.22
6	c	1	58.95	58.45	57.42	57.9	57.5	55.58	54.23
6	c	2	58.95	58.25	57.67	57.9	57.19	56.16	54.38

Table 4 continued

7	a	1	60.5	60.21	60.47	60.7	58.87	57.53	56.51
7	a	2	60.7	59.27	60.05	60.7	58.83	57.51	56.48
7	b	1	60.6	60.65	59.44	60.1	58.75	57.43	56.44
7	b	2	60.4	60.22	59.64	60.7	58.76	57.7	56.29
7	c	1	60.2	60.4	59.72	59.9	58.7	57.15	56.23
7	c	2	60.2	60.3	59.72	60.1	58.72	57.09	56.31
8	a	1	58.15	58.16	57.24	57.1	57.01	54.89	53.92
8	a	2	58.55	58.12	56.98	57.7	56.7	54.67	53.96
8	b	1	58.35	57.44	57.44	56.9	56.73	54.83	53.91
8	b	2	58.55	57.66	57.56	57.5	56.83	55.05	54.16
8	c	1	58.05	57.38	57.39	57.9	56.47	54.73	53.96
8	c	2	58.45	57.93	57.42	58.3	56.48	54.98	53.81
9	a	1	60.1	59.86	59.86	60.7	58.68	56.78	56.43
9	a	2	60.2	59.26	59.72	60.5	58.65	56.91	56.34
9	b	1	60.3	59.8	60.07	61.1	58.1	57.38	56.09
9	b	2	60.7	59.76	59.99	61.1	58.94	57.88	56.29
9	c	1	60.8	60.21	60.25	60.7	58.99	58.14	56.86
9	c	2	60.8	60.31	60.46	60.7	59.15	58.4	56.77
10	a	1	48.65	49.37	47.09	49.8	49.07	46.75	46.04
10	a	2	49.85	47.34	48.1	49.6	49	46.4	45.51
10	b	1	48.35	47.48	47.86	49.28	48.43	47.56	45.48
10	b	2	48.45	47.2	48.22	49.2	48.56	47.48	45.88
10	c	1	48.65	49.28	47.37	49.6	47.8	46.6	45.59
10	c	2	48.75	47.72	47.94	49.2	49.97	46.51	45.58

Table 5 Hectolitre mass measurements for the 10 repetitions on each of the three samples Experiment 4: comparison between two German and two South African HLM devices using oat sub-samples.

Sample	Germany 1	Germany 2	SA 1	SA 2
1	54.90	54.50	50.06	52.37
1	54.80	54.70	51.70	52.22
1	54.10	54.80	51.67	50.58
1	55.00	54.70	50.68	51.38
1	54.80	55.20	51.82	50.57
1	55.10	55.20	51.58	51.83
1	55.20	54.80	51.26	51.28
1	54.80	54.90	50.19	51.85
1	54.70	54.80	50.94	51.94
1	54.40	55.00	52.28	51.10
2	51.35	51.35	48.85	48.36
2	50.75	50.95	48.89	48.55
2	51.35	51.15	46.90	47.19
2	51.25	50.95	48.54	48.73
2	50.85	51.15	48.53	47.87
2	51.45	51.35	47.55	48.31
2	51.80	51.45	48.07	48.18
2	51.60	51.05	48.29	47.91
2	50.95	51.35	48.35	47.75
2	51.05	51.25	48.00	48.29
3	58.25	58.25	55.19	55.52
3	58.25	58.25	55.55	55.36
3	57.65	57.65	55.03	55.64
3	57.85	57.85	55.44	55.48
3	58.65	58.65	55.52	54.62
3	58.05	58.05	55.13	55.00
3	58.15	58.15	55.31	55.28
3	58.25	58.25	54.98	55.47
3	58.05	58.05	55.39	55.33
3	58.25	58.25	55.38	55.34

Table 6. Hectolitre mass (HLM) measurements for the 10 repetitions on each of the three oat samples before and after rubbing. respectively. Experiment 5: effect of rubbing of oats on the HLM values using sub-samples.

			HLM before rubbing				
Sample	**Germany**	**Australia**	**UK**	**Canada**	**France**	**SA**	**USA**
1	56.25	55.89	56.95	56.1	55.14	53.89	53.28
1	56.25	55.52	56.35	56.5	55.41	54.48	53.97
1	56.45	56.63	55.75	56.5	55.02	53.85	53.87
1	56.25	55.62	56.15	55.9	55.05	53.62	53.92
1	56.65	56.22	55	56.5	55.73	54.42	53.79
1	56.15	56.07	55.21	56.3	55.58	53.66	53.6
1	56.05	56.16	55.36	56.5	55.8	53.75	53.4
1	56.15	55.11	56.12	56.1	55.5	53.83	53.27
1	56.15	56.46	55.99	56.9	55.48	53.47	53.78
2	51.15	50.95	50.17	51.4	50.01	47.91	48.15
2	51.15	51.8	49.55	51.6	50.01	48.06	47.68
2	51.05	50.31	50.47	51.4	50.04	48.56	47.86
2	51.05	50.82	50.53	51.8	50.01	48.69	48.33
2	51.25	49.44	49.68	51.4	49.88	48.27	48.03
2	51.6	50.4	49.89	51.6	49.64	48.66	48.22
2	51.45	50.55	50.35	51.4	49.92	48.49	47.91
2	51.25	50.06	50.44	51.8	49.84	48.65	47.98
2	51.25	50.58	49.9	51.8	49.99	48.96	48.05
2	51.15	50.89	49.69	51.8	49.89	48.59	48.12
3	57.05	56.49	56.45	57.3	55.74	54.87	53.41
3	57.05	56.63	56.29	57.1	56.05	54.98	53.21
3	57.05	56.25	56.65	57.3	55.02	54.46	53.47
3	57.15	56.58	56.52	56.7	56.2	54.7	53.78
3	57.45	56.04	55.61	57.5	56.16	54.63	53.83
3	57.05	56.06	55.63	56.5	56.16	55.83	53.64
3	56.95	56.15	56.64	57.1	56.34	55.02	53.36
3	56.95	56.39	56.77	57.5	56.06	55.27	53.21
3	57.05	56.43	56.7	56.1	56.38	54.91	53.27
3	56.85	56.06	56.51	57.7	56.26	54.48	53.46

Table 6 continued

	HLM after rubbing						
Sample	**Germany**	**Australia**	**UK**	**Canada**	**France**	**SA**	**USA**
1	58.25	57.28	57.84	58.3	56.01	54.5	54.57
1	58.05	57.28	57.46	58.1	56.23	55.01	54.12
1	57.65	57.16	57.32	57.7	56.84	54.78	54.34
1	58.75	57.39	56.91	57.9	56	54.68	54.34
1	58.05	57.83	57.51	58.5	56.48	54.7	54.43
1	57.85	58.53	57.78	57.7	56.08	54.95	54.46
1	57.55	57.01	57.67	57.7	56.67	55.01	54.37
1	58.35	57.12	57.64	58.3	56.18	54.89	54.39
1	57.85	57.73	57.48	58.3	56.32	55.51	54.16
1	57.95	58.27	57.14	57.7	56.35	55.3	54.41
2	52.4	52.44	51.51	52.6	51.33	49.8	48.89
2	52.5	51.78	51.21	52.8	51.24	49.82	49.13
2	52.4	51.27	51.19	52.4	51.31	49.74	49.14
2	52.2	51.15	51.23	52.4	51.04	49.69	48.92
2	52.1	51.32	51.14	52.8	51.02	49.84	48.84
2	52.4	51.43	51.45	52.6	50.88	49.96	48.94
2	52.5	51.45	51.26	52	50.61	50.68	48.99
2	52.3	51.8	51.37	52.6	50.85	50.47	48.86
2	52.2	51.64	51.2	52.2	51.37	50.19	49.05
2	52.3	51.95	51.28	52.4	50.84	50.8	49.1
3	58.75	57.56	57.65	58.5	56.94	55.75	54.2
3	58.85	57.22	57.74	58.1	56.95	55.87	54.25
3	58.45	57.5	57.84	58.1	56.82	56.09	54.77
3	58.05	57.51	57.17	57.9	56.8	56.55	54.33
3	58.25	57.43	57.14	57.7	56.98	55.63	54.54
3	58.85	58.64	57.2	57.7	57.32	56.27	54.77
3	58.65	57.43	57.19	57.7	56.98	55.82	54.63
3	58.25	57.09	57.7	58.3	57.21	55.92	54.3
3	58.15	57.15	57.47	58.1	57.1	56.26	54.69
3	58.45	57.05	56.92	58.5	57.11	55.76	54.52

Table 7 Hectolitre mass (HLM) values for ten measurements on each sample using work samples of 5 oat samples. Experiment 6: effect of operator on HLM determinations.

		Operator 1		**Operator 2**		**Operator 3**	
Sample	**Rep.**	**SA 1**	**SA 2**	**SA 1**	**SA 2**	**SA 1**	**SA 2**
1	1	54.62	54.66	54.77	54.88	54.5	56.13
	2	55.33	54.53	54.88	55.08	55.14	55.23
	3	54.97	54.63	55.02	55.08	55.59	55.39
	4	55.01	54.27	54.93	54.99	55.13	55.1
	5	54.51	54.93	55	54.94	54.91	55.1
	6	54.57	54.85	54.8	54.89	55.08	54.78
	7	54.35	54.83	54.95	55.02	56.41	54.95
	8	54.59	54.69	54.78	54.85	55.32	54.44
	9	54.56	55.03	54.99	55.08	55.1	54.67
	10	54.42	54.9	55.38	55.12	54.91	55.26
2	1	52.00	51.91	51.94	52.31	52.39	51.94
	2	52.76	52.55	51.92	52.25	52.82	52.44
	3	52.34	52.38	52.06	52.2	52.66	53.34
	4	52.57	52.2	52.08	52.08	52.28	52.29
	5	52.38	52.95	52.27	52.28	52.22	52.47
	6	51.95	52.07	52.25	52.22	52.88	52.46
	7	52.25	52.69	52.27	52.28	51.91	51.47
	8	52.75	52.25	52.28	52.07	52.92	53.29
	9	52.34	52.90	52.21	52.14	52.56	53.26
	10	53.04	52.4	51.98	52.58	53.05	52.36
3	1	55.13	54.78	55.27	55.14	55.4	56.66
	2	54.69	55.06	55.48	55.18	55.23	56.4
	3	54.79	54.78	55.11	55.21	55.21	55.75
	4	55.02	55.16	55.17	55.14	55.45	56.38
	5	54.77	55.08	55.16	55.29	55.33	55.51
	6	54.7	55.00	55.28	55.17	55.74	55.4
	7	54.63	54.51	55.27	55.3	56.15	56.01
	8	54.94	54.87	55.26	55.2	55.54	55.87
	9	54.79	54.9	55.33	55.35	55.65	55.55
	10	55.15	55.06	55.32	55.2	54.84	55.69
4	1	48.7	48.49	48.84	48.6	49.61	49.58
	2	48.22	48.49	48.22	48.55	49.52	49.23
	3	48.07	48.46	48.37	48.6	49.63	49.57
	4	48.1	49.91	48.77	48.69	49.4	50.04
	5	48.15	49.03	48.74	48.61	49.08	49.54
	6	48.31	48.83	48.35	48.81	49.28	49.46
	7	48.52	48.1	48.6	48.6	50.64	49.84

Table 7 continued

	8	48.38	48.48	48.65	48.94	49.34	49.52
	9	48.6	48.46	48.67	48.67	49.8	49.87
	10	48.67	48.51	48.82	48.67	49.95	49.39
5	1	46.41	46.42	46.15	46.28	47.42	46.94
	2	46.62	46.59	46.2	46.54	47.26	46.47
	3	46.54	46.6	46.38	46.6	46.81	46.68
	4	46.42	46.9	46.41	46.43	47.94	46.99
	5	46.57	46.55	46.29	46.45	47.6	47.13
	6	46.55	46.88	46.22	46.56	47.69	47.54
	7	46.67	47.01	46.56	46.5	47.97	47.57
	8	46.37	47.09	46.49	46.59	47.63	47.75
	9	46.65	46.45	46.35	46.39	46.86	47.57
	10	47.02	46.83	46.25	46.33	47.33	46.57

Table 8 Hectolitre mass (HLM) values of oat samples after a number of wetting and drying cyles. Experiment 6: effect of consecutive wetting and drying of oat samples on HLM results.

Sample	Moisture	Rep	Germany	Australia	UK	Canada	France	SA	USA
Original moisture content									
1	11.5	1	59.9	59.83	59.89	60.1	58.58	57.23	56.34
		2	60.2	59.29	59.43	60.3	58.73	57.04	56.43
2	10.8	1	56.95	56.39	56.82	57.9	55.81	53.98	53.09
		2	57.65	56.8	56.02	56.7	55.59	53.37	53.32
3	11.3	1	48.65	48.8	48.08	48.4	47.21	45.99	45.23
		2	48.55	47.8	48.1	48.4	47.2	45.7	45.47
4	10.9	1	55.95	55.89	55.97	56.7	54.72	53.74	52.67
		2	56.05	54.99	55.32	56.5	54.92	52.73	52.71
Original to 14 % moisture content									
1	13.5	1	58.65	58.68	58.2	58.9	57.05	56.04	54.58
		2	58.65	57.97	58.44	59.3	57.12	56.66	54.46
2	13.7	1	54.40	53.5	53.74	54	53.03	52.1	50.63
		2	54.50	53.93	54.06	54.6	53.18	52.15	50.91
3	13.3	1	47.10	46.7	46.45	47.8	45.69	44.58	43.44
		2	47.30	46.87	46.39	47.4	45.8	44.74	43.39
4	13.9	1	53.10	52.05	53.14	53.2	51.96	51.38	49.52
		2	53.00	52.25	52.14	53.8	51.68	51.03	49.14
Original to 16 % moisture content									
1	15.3	1	57.56	57.31	57.4	57.5	56.3	55.62	53.51
		2	57.85	57.27	57.08	57.7	56.44	55.02	53.62
2	15.8	1	52.6	51.87	51.63	52.8	51.05	49.65	48.67
		2	52.8	51.89	52.26	52.6	50.88	49.16	48.54
3	16.1	1	45.4	44.41	44.95	45	43.23	41.97	41.47
		2	45.8	44.3	44.76	45.2	43.46	42.4	41.45
4	15.1	1	51.6	51.29	50.83	51.6	49.95	48.94	47.49
		2	51.6	50.88	51.38	51.6	50.82	49.81	47.66
Original to 18% moisture content									
1	17.6	1	55.3	54.77	54.49	55.9	53.74	52.36	51.33
		2	55.45	55.21	54.52	56.3	53.94	53.16	51.63
2	17	1	50.55	49.81	50.63	50.4	49.07	47.53	46.7
		2	50.65	49.69	50.4	50	48.97	47.61	46.73
3	16.9	1	43.05	42.66	42.83	43	41.74	40.58	39.02
		2	43.25	42.37	42.52	43	41.68	40.38	39.29
4	17.5	1	49.55	49.26	48.96	50	48.33	46.72	45.6
		2	49.75	49.03	48.72	50	47.81	47.81	45.98

Table 8 continued

Sample	Moisture	Rep	German	Australia	UK	Canada	France	SA	USA
Original moisture content to 10 % moisture content									
1	9.7	1	60.6	60.23	59.98	60.9	58.96	57.68	57.26
		2	60.8	59.99	59.87	60.7	58.91	57.94	56.79
2	9.4	1	57.65	56.94	56.29	58.1	55.83	54.01	53.26
		2	57.85	56.49	56.84	57.9	55.89	54.36	53.45
3	10.1	1	49.75	49.25	48.95	49.9	48.56	47.78	46.43
		2	49.95	49.09	48.73	49.8	48.39	47.33	46.82
4	10.2	1	56.82	55.99	56.02	56.9	54.75	53.82	52.85
		2	56.45	56.27	55.41	56.6	54.96	53.55	52.77
14 % moisture content to 10 % moisture content									
1	9.2	1	58.95	58.05	58.86	59.5	58.32	56.82	56.09
		2	59.3	58.53	58.56	58.9	58.23	57.32	55.78
2	9.8	1	55.75	54.83	54.81	55.4	54.14	52.79	51.89
		2	55.85	55.09	55.19	55.6	54.22	53.05	51.9
3	9.8	1	48.95	48.5	48.89	49.4	48.1	46.3	45.89
		2	49.15	48.37	48.71	49.8	47.87	46.05	45.86
4	9.7	1	55	54.32	54.53	55.2	54.08	52.11	51.91
		2	55.1	55.06	54.26	55.6	53.93	52.72	51.66
16 %moisture content to 10 % moisture content									
1	10.1	1	59.3	58.7	58.02	59.5	58.11	56.38	55.6
		2	59.05	58.49	58.38	59.1	57.9	57.1	55.44
2	9.9	1	55.85	54.38	55.53	55.2	54.35	51.98	51.67
		2	55.95	54.89	55.3	55.4	54.44	52.17	51.6
3	10.2	1	48.35	47.76	48.26	48.2	47.49	45.96	45.2
		2	48.45	47.72	48.27	48.6	47.34	46.09	45.37
4	10.2	1	54.3	53.73	53.88	54	53.53	52.71	51.23
		2	54.3	54.03	54.28	54.4	53.42	52.06	51.39
18 %moisture content to 10 % moisture content									
1	10.9	1	58.75	58.8	57.93	59.1	57.62	55.92	55.6
		2	58.55	57.88	58.42	58.9	57.61	56.02	55.31
2	9.8	1	55.1	54.43	54.72	55	53.89	51.82	51.34
		2	55.2	54.25	53.84	54.8	53.66	51.90	51.17
3	10.6	1	48.55	47.95	47.82	49	47.46	46.07	45.09
		2	49.05	47.59	47.98	49.2	47.45	46.06	45.14
4	9.9	1	54.2	54.99	54.39	55.2	53.32	51.73	51.39
		2	54.2	53.69	54.18	55	53.15	51.99	51.52

Table 8 continued

Sample	Moisture	Rep	Germany	Australia	UK	Canada	France	SA	USA
10 % moisture content to original moisture content									
1	11.2	1	59.8	59.03	59.36	59.9	58.17	56.97	55.93
		2	56.7	59.29	58.91	59.5	58.1	56.95	56.09
2	10.4	1	56.95	56.2	55.83	56.7	54.38	53.03	52.68
		2	56.85	55.93	55.54	56.5	54.78	53.38	52.66
3	11.5	1	48.85	48.3	47.98	48.2	47.26	45.69	45.22
		2	48.65	48.01	48.2	48.6	47.13	45.17	45.23
4	10.5	1	56.15	54.92	55.55	56.3	54.52	52.16	52.53
		2	55.95	55.28	55.41	55.9	54.43	52.23	52.37
10 % moisture content to 14 % moisture content									
1	14.2	1	58.05	57.48	56.97	58.3	56.6	55.29	54.2
		2	57.75	57.32	56.81	58.3	56.46	55.33	54.21
2	13.8	1	54.4	53.08	54.09	55	53.64	52.44	51.14
		2	54.6	53.49	54.48	54.4	54.02	51.76	51.09
3	13.7	1	47.5	46.36	46.6	47.2	45.48	43.83	43.75
		2	47.5	46.62	46.87	47.4	45.99	44.49	43.43
4	14.4	1	53.5	52.04	53.06	54	52.01	49.95	49.52
		2	53.5	52.53	52.94	53.4	51.99	50.11	49.17
10 % moisture content to 16 % moisture content									
1	15.6	1	56.05	56.5	55.49	56.7	55.26	53.8	52.9
		2	56.05	56.03	55.93	57.1	55.45	53.66	52.97
2	15.3	1	51.7	50.23	50.83	51.8	50.25	48.19	47.66
		2	51.8	50.65	50.56	52	50.39	49.15	47.53
3	15.8	1	44.3	43.57	43.26	44.4	42.61	41.38	40.13
		2	44.3	42.98	43.05	44	42.64	41.58	40.49
4	15.2	1	51.15	50.08	50.21	51.4	49.17	48.35	47.49
		2	51.25	50.05	50.35	51.4	49.23	48.44	47.45
10 % moisture content to 18 % moisture content									
1	17	1	54	53.9	53.21	54.4	52.51	51.32	50.73
		2	53.9	54.18	53.34	54.8	52.44	51.79	50.44
2	17.4	1	50.15	49.2	49.3	49.6	48.54	47.14	46.02
		2	50.15	48.66	49	50	48.38	46.85	46.07
3	17.2	1	42.35	41.64	41.18	41.8	40.69	39.79	37.92
		2	42.45	41	41.92	42.2	40.95	39.95	38.18
4	17.6	1	49.15	49.28	48.74	49.2	47.93	46.79	45.62
		2	49.35	48.47	49.09	49.6	48.14	47.07	45.97