DEVELOPMENT OF SAFE STORAGE GUIDELINES FOR PRAIRIE-GROWN FLAXSEED (*Linum usitatissimum*) AND CHARACTERIZATION OF STORED SEEDS USING NEAR INFRARED SPECTROSCOPY

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THESIS ABSTRACT

Canada is the largest producer of flaxseed in the world with a production of 483.2 thousand tonnes of flax with seeded area of 376 thousand hectares in 2019. Maintaining the quality of huge amounts of seeds especially under constantly varying environmental conditions is an ongoing challenge. Therefore, the current research focuses on developing safe storage guidelines for flaxseed, which can help farmers plan proper post-harvest operations to avoid quality and quantity losses of stored flaxseeds. Flaxseed was locally sourced from a Manitoba farm and was conditioned to moisture contents of 7, 8, 9 and 13% and stored in relative humidity conditions of 54, 65, 75 and 94% at temperatures of 10, 20, and 30℃ for 16 weeks. The indices of spoilage were seed germination, free fatty acid value (FAV), visible mold, and protein content. The influence of storage factors such as temperature, relative humidity and storage period on germination rate and FAV was statistically significant (P = 0.05). No significant difference was observed in the protein content with relative humidity, but significant change was observed with temperature. It was found that samples at 10 and 20 ℃ can be safely stored for at least 16 weeks provided that moisture content is maintained between 7 and 9 %. The seeds must be dried within 3 weeks of storage, if it is to be stored at a moisture content 13 % or above and at temperatures over 20 ℃. To retain good quality and maintain seed viability during storage for extended periods, temperature should be maintained below 20℃.

The quality of flaxseed changes when exported to warmer countries which might deteriorate the oil quality. Moreover, due to increase in demands and export, the current food processing business requires rapid, precise, ecologically sustainable, and cost-effective techniques to assess oilseed quality. Therefore, another goal of this study is to use non-destructive imaging techniques of visible near-infrared (Vis-NIR; 450-1100 nm) and shortwave infrared (SWIR; 1000-2500 nm) hyperspectral imaging for flaxseed quality assessment based on storage condition and duration. Principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and partial least squares regression (PLSR) models were developed to model flax quality. Satisfactory groupings were demonstrated through PCA models in the Vis-NIR range based on storage period and temperature, but PCA in the SWIR range distinguished flaxseed based on initial moisture content. The PLS-DA model achieved classification accuracies of 81.5, 72.8, and 87.5 % in

calibration, cross-validation, and external prediction for flaxseed based on initial MC, respectively. The PLSR prediction model for MC yielded an R_c^2 of 0.96, R_{cv}^2 of 0.95 and R_p^2 of 0.97. PLSR models did not yield promising results when it came to predicting protein content and germination. As a result, it was established that NIRS may be utilised by food processors and farmers to analyse flaxseed quality non-destructively and make logistical decisions.

Keywords: Flaxseed, relative humidity, temperature, moisture content, visible mold, FAV, germination, storage guidelines.

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Chapter 1- Overview of the thesis

1.1. Background

Flax (*Linum usitatissimum* L.) is an important oilseed for industrial, food and feed use. Etymologically, the specific epithet of the specie's Latin name means "very useful", which establishes its importance since antient times. Around 7,000 years ago, the plant was domesticated. The first European settlers introduced flax in Canada and North America and has been cultivated ever since. It was first grown in Quebec in 1617 from where it spread throughout the continent with settlers in the 1800s (Ehrensing 2008). Flaxseed stands out among the oilseeds owing to its high levels of α-linolenic acid (ALA, 18:3n-3) and lignans. The seeds contain 35-45% oil, with ALA accounting for 45-52% of the composition (Singh et al. 2011). Constitutionally, it contains about 28–30% protein and 35% fibre (Kajla et al., 2015). Oil derived from the seed is widely used as an industrial drying oil (e.g., in paints, steelworks, linoleum and oilcloths). Flax was certified as beneficial to human health by Health Canada in 2014, with its consumption linked to decrease blood cholesterol levels. Edible oil is processed from flaxseed oil that contains low linolenic acid and high linoleic acid (Singh et al. 2011). It may be consumed raw or cooked, and it can be found in salads, bread, muffins, spaghetti, and energy snack bars (Kajla et al., 2015).

Flax is planted on approximately 2.6 million hectares across the world (Charney 2014). Since 1994, Canada has been the leading flax producer, followed by China, United States of America, India, and the European Union. According to Statistics Canada, 483.2 thousand tonnes of flaxseed were produced on 376 thousand hectares in 2019. In Canada, the seeds are sown in April or May and harvested when the plants reach the optimal maturation stage between September and October (Mishra and Awasthi 2021). New varieties have been produced as a result of breeding initiatives to adapt the crop to various climatic and geographic circumstances, particularly in the Canadian Prairies.

In 1900's, three major breeding programs were initiated: the Agriculture and Agri-Food Canada (AAFC) program located at the Cereal Research Centre in Morden, Manitoba; the Crop Development Centre program located at the University of Saskatchewan and the Saskatoon R and D facility of Crop Production Services Canada Inc., in Saskatoon, Saskatchewan. Disease resistance to rust and wilt were emphasised in these programmes. Farmers in Manitoba are

becoming more interested in cultivating flaxseed, putting the province in third place after Saskatchewan and Alberta as Canada's largest flaxseed producer (Flax Council of Canada, Statistic 2014-2015). It is necessary to develop ideal growing, post-harvest, and storage conditions in order to cultivate and deliver high quality of this valuable oilseed to the market. To avoid quality-related economic losses, proper handling and safe storage are critical (Yousif 2014).

The safe storage period is determined based on the moisture content and temperature of the seeds during harvest. The quality of the stored grain crops can significantly be impacted by factors such as seed maturity, moisture content, storage temperature, storage period, moulds, insects, mites, dockage, environment, and postharvest practices (Jayas 1995). Weather patterns during harvest might result in excessive moisture levels in the seeds, making it unsuitable for safe storage. Agricultural goods are vulnerable to degradation, mould spoilage, insect infestation, and self-heating during storage (Obaldo 1991). Even when seeds are kept at an acceptable moisture content, fluctuations in temperature and relative humidity inside a bin can cause biochemical changes that compromise their quality. Oilseeds, unlike cereal grains, have a higher risk of self-ignition. Temperature gradients that occur as a result of temperature variations between seeds near the walls and seeds in the bulk, generate continuous moisture gains and losses in stored grains. Moisture build-up can occur as a result of these gradients, resulting in hotspots or high moisture pockets. Conditioning newly harvested damp warm seeds is a critical step in ensuring optimum storage period. The process involves air movement through a grain bulk to ensure that it can be stored for an extended length of time without losing quality.

According to Muir (2001), the rate of germination, free fatty acid value (FAV), mould growth, physical appearance, and nutritional value must be assessed on a continual basis to evaluate the condition of the stored grain. At high temperatures, high moisture grain may need to be dried and cooled faster than low moisture grain at lower temperatures.

Freshly harvested grain can only be stored for a limited time period during which post-harvest operations such as conditioning, turning etc. can be done to ensure long term storage. The amount of this limited time period available to conduct postharvest operations significantly depends on grain moisture content and temperature at the harvest stage. As a result, standards for all common grains at all conceivable moisture contents and temperatures must be developed to provide farmers with information on the number of days available to complete post-harvest operations before grain

degradation starts. Storage guidelines have been developed for canola (Sun et al. 2014), wheat (Nithya et al. 2011), red lentils (Cenkowski et al. 2015), soybeans (Diaz-Contreras et al., 2021), etc. As per our knowledge, only two research works have established safe storage guidelines for frequently farmed flaxseed varieties in North America (White et al. 1999, White and Jayas 1991). But due to continuous variations in the climatic conditions in the Canadian Prairies and development of new varieties, it is imperative to update these safe storage guidelines. Therefore, the major objective of this research work was to determine appropriate storage guidelines for flaxseed cultivated in the Canadian Prairies.

Systematic investigation for flaxseed quality attributes based on storage conditions and storage time are critical especially for oil processing and export. The reduction in end product quality can be significantly accelerated if seeds are damaged or wet during harvest, handling, and storage owing to increased moisture content (MC). Yet no standardization for the classification of flaxseed based on storage conditions and time for quality estimation has been developed. To meet this need, the current study used spectral data extracted from near visible-near-infrared (VNIR) and shortwave-infrared (SWIR) hyperspectral imaging to predict quality parameters such as moisture content and FAV. Combining spectral data with multivariate data analysis tools can help characterise food products in a rapid, reliable, and cost-effective manner (Babellahi et al. 2020; Chaudhry et al. 2018). Hyperspectral imaging, which combines imaging and spectroscopic techniques, can determine the spatial distribution and spectral dimension of a chemical compound (Elmasry et al. 2012). These methods have been demonstrated to reliably predict the chemical composition of green lentils (Kaliramesh et al. 2013), and isoflavones concentration in soybeans (Kezhu et al. 2014). Therefore, this technique can be used effectively for evaluating the quality of food products.

1.2. Thesis objectives

- To develop safe storage guidelines for Prairie grown flaxseed.
- To utilize spectral signals in the Vis-NIR (400-1000 nm) and SWIR (1000-2500 nm) regions to develop classification and prediction models for the non-destructive estimation of stored flaxseed quality.

1.3. Structure of the thesis

This thesis is comprised of five chapters. Chapter 1 provides introduction to the research and its objectives. Chapter 2 provides a comprehensive literature review. Chapters 3 and 4 discuss the objectives, experimental designs, and obtained results of the research in a manuscript-based format. Chapter 3 is titled "Development of Safe Storage Guidelines for Prairie-grown Flax" is submitted in Journal of Stored Products Research and Chapter 4 as "Characterization of stored flaxseed using near infrared spectroscopy" submitted in Journal of Food Measurement and Characterization. The thesis summary, overall conclusions and future recommendation are included in chapter five.

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Chapter 2-Literature review

2.1. Flaxseed

Flax (*Linum usitassimum*) is a blue flowering annual herb that produces tiny flat seeds that range from golden yellow to reddish brown in color. It belongs to the Lineaceae family. The five-petaled flowers create a five-celled boll that can bear up to ten seeds. Although flax is typically planted in the spring, a few winter-hardy cultivars have been produced that may be planted in the fall (Ehrensing 2008).

The oil extracted from flax can be termed as flaxseed oil or linseed oil. The difference lies in its usage. Flaxseed oil is a term used to describe when the oil is fit for human consumptions, whereas the term ‘linseed oil’ is used when the oil extracted is utilized for industrial purposes (Goyal et al. 2014). Because of its usage as a common crude material for contemporary purposes, flax has piqued the interest of industry, horticulture, and growers in recent years. As mentioned in previous sections, flaxseed is gaining importance as an oilseed due to the presence of approximately 34-45% oil content, 28-30% protein and 35% fibre. Around 35-45% of the total oil content in flaxseed comprises of Alpha-linolenic acid (ALA) (Mishra and Awasthi, 2021). When the oil is exposed to air, the double bonds of ALA react with oxygen, forming a soft, long-lasting coating. This property is referred to as "drying" quality. Because flaxseed oil contains more than half of linolenic acid, it is suitable for current usage in protective coatings such as polishes, lacquers, stains, and paints. Omega-3 unsaturated fats have long been recognized for their beneficial effects on human health. The word "solin" was coined by the Flax Council of Canada to describe flax cultivars that contain less than 5% ALA and are suited for use in the food industry (Jhala and Hall 2010). Flaxseed is an edible seed that may be consumed raw or cooked. The appealing, reddish-brown-colored oval seeds of flax give a captivating nutty flavor to salads, bread, muffins, spaghetti, and energy snack bars, as well as excellent nourishment (Kajla et al., 2015). The high protein meal is utilized as animal feed once the flax oil is extracted. (Singh et al. 2011).

2.2. Flaxseed grading

Oilseed quality standards have been established in several nations. These regulations classify oilseeds according to various characteristics, making it easier for customers to choose products

that meet the specific requirements. For oilseeds, two types of grading systems are widely employed. The first method is to sort oilseeds by numerical grade, as done in Canada, Sweden, and the United States. This approach allows each grade to have its own set of qualities. The second is based on individual qualities. Australia and the European Economic Community employ this approach (EEC). Both aforementioned grading systems require the assessment of degrading factors (Daun and Burch 1984). Daun and Burch (1984) thoroughly described Canada's oilseed grading system, as well as the factors that might affect oil quality and its correlation. The quality of oilseeds is deemed to have deteriorated when (a) the oil or protein content of the seed has diminished, and (b) the oil content and meal of flaxseed are difficult to convert into desirable products. Normally, laboratory and visual assessments are used to assess degrading factors. Oil content, protein content, chlorophyll, and FAV determination are all standardized laboratory tests. Flaxseed is mostly degraded by immaturity, heat, broken, and sprouting seeds (Daun and Burch, 1984). As a result, in the early 1900s, three major breeding programmes were established to develop flax varieties for Canadian Prairies: Agriculture and Agri-Food Canada (AAFC) program located at the Cereal Research Centre in Morden, Manitoba; the Saskatoon research and development facility of Crop Production Services Canada Inc. (CPS). In addition to disease control, these programmes emphasised disease resistance against rust and wilt.

2.3. Flaxseed storage

The interplay of the biotic (grain, insects, and mould) and abiotic (temperature, relative humidity, sun radiation, gas concentration) factors in grain storage structures (grain bins, silo bags, and bunkers) factors have a key influence in crop quality variations during storage (Sinha and Muir 1973). Flaxseed, like crops and other oilseeds, can degrade in quality if stored under unfavorable conditions. The seed's moisture content and temperature impact the events that occur during storage, which might result in spoiling and self-heating. Wet harvesting, moisture movement in bulk-stored seeds, and moisture seepage through storage structure breaches can all cause flax moisture levels to increase over safe storage limits, allowing microflora to grow and insects to infest the seed. Flaxseed should have a moisture level of less than 10% before storage, according to Ehrensing (2008). Seed drying is critical for achieving a lower moisture content. Proper aeration can help seeds attain the correct moisture content. Flax and other high-oil seeds deteriorate more

quickly in storage. The air flow in storage bins containing small sized seeds like flax can be obstructed due to less void volume, or interstitial space between the stored mass of seeds.

2.4. Quality assessment parameters

2.4.1. Moisture content

The amount of moisture in seeds is defined as the moisture content. Availability of unbound water might increase if samples are stored in a humid environment. This availability of unbound water to the seed might result in a variety of changes, such as moisture migration, precipitate penetration, and increase in initial moisture content, may occur (Chelladurai 2016). A hot spot can form in the grain mass because of the higher moisture content, which is conducive to the growth of insects, mites, and microorganisms, all of which contribute to grain degradation. As a result, grain stored at high moisture levels are more likely to deteriorate sooner than grain stored at low moisture levels. According to studies, lower moisture contents allowed the grain to be stored for longer periods of time with no signs of degradation (Sun 1999; Zomorodian et al. 2011). Sun et al. (2014) stored canola with an initial moisture content of 8, 10, 12 and 14% at 10, 20, 30 and 40 °C. Canola stored at higher moisture content resulted in a further increase in moisture content. Canola that was kept at a 10 °C showed less changes. According to Gunasekaran (2006), after 16 weeks of storage at 20, 30, and 40 °C, the moisture content of canola steadily decreased from initial moisture contents. The final moisture content of 7.5 and 10.0% initial moisture canola was 3.2 and 2.6 %, respectively when stored at 40 °C. By the end of the study, all the samples stored at 40 °C had a moisture level of less than 6%, making it nearly impossible to correlate the results with the canola's storage moisture content. Mills and Sinha (1980) compared the safe storage time of different moisture content rapeseeds stored in the laboratory at different temperatures to rapeseed stored in non-aerated farm bins for 5 months and found that rapeseed with 8.5% or less moisture content could be stored in non-aerated bins for 5 months without spoiling. Small measurement errors in the critical moisture range can mean the difference between spoilage or safe storage.

2.4.2. Free Fatty acid value

Another indication of grain quality degradation is the free fatty acid value (FAV). They are produced because of enzymatic secretion by microorganisms in grain, which hydrolyze the lipids. The grain's nutritional value is reduced because of this biochemical change (Sathya et al. 2009). Free fatty acids are measured in mg of potassium hydroxide (KOH) necessary to neutralise the free fatty acids contained in 100 g of moisture-free grain. Because of the direct decrease in oil quality in oilseeds, the increase in FAV is more significant. The value of free fatty acids is also linked to an increase in fungal growth and respiration (Mills and Sinha 1980). According to Christensen and Kaufmann (1969), a rise in mould population has a positive correlation with FAV and CO_2. However, the FAV does not have an absolute threshold that correlates with the amount of degradation of a sample; instead, only relative changes are utilised to measure the deterioration. White et al. 1998 studied the quality changes in flaxseed with moisture levels of 8, 9.5, 11, and 12.5% (wet basis) kept at 10, 15, 20, 25, 30, and 35 °C for 20 weeks. They reported that when the moisture content, temperature, and time over 6 months increased, so did the amounts of free fatty acids. Jian et al. (2012) observed that canola with high oil content has lower thermal conductivity and diffusivity which might affect the temperature migration during storage and cause hotspot generation. Sathya et al. (2009) stored canola with four different moisture contents, 7.5, 10.0, 12.5, and 15.0 % (wet basis) at 10, 20, 30, and 40 °C for 16 weeks. They found that when the moisture content of the canola and the storage temperature increased, the FAV value increased. For dry canola (7.5% moisture content) held at low temperature, the rate of increase of FAV was acceptable (less than 1.5-fold increase), while for high moisture seeds (15% MC), FAV increased more than 3-fold after 3 weeks of storage at high temperatures (30 and 40 °C). Sun et al. (2014) studied the quality variations in high and low oil content canola cultivars with moisture levels of 8, 10, 12 and 14% (wet basis) kept at 10, 20, 30 and 40 °C for 20 weeks. FAV of canola stored at 10, 20, and 30 °C increased with storage time. FAV of canola stored at 40 °C increased up to 6 weeks of storage before dropping dramatically, and they speculated that the oxidation of free fatty acids at higher temperatures could be the reason for this drop. When storing durum wheat and rye at 10, 20, 30, and 40 °C, Nithya et al. (2011) and Rajarammanna. et al. (2010) found a similar pattern of increasing FAV with increasing storage duration.

2.4.3. Protein content

Nutritional changes can be evaluated by the change in the protein content. Cotyledons of the seed contain most of the protein composition (Kajla et al. 2015), including globulin (26–58 %) and albumin (20–42 %). Hall et al. (2006) found that flaxseed proteins exhibit antifungal properties against *Alternaria solani, Candida albicans* and *Aspergillus flavus.* Flaxseed protein combined with varied amounts of polysaccharide gums have been explored as additives, emulsifiers, and stabilizers in the food industry (Oomah and Mazza 1993). Effect of storage conditions on protein content of red lentils was studied by Sravanthi et al., (2013). No significant difference was reported throughout the storage of 16 weeks. Rani et al., (2013) reported a significant difference in protein content and moisture content.

2.4.4. Seed Germination rate

Seed germination rate is one of the most important aspects to be considered during storage. As a result, this component is critical in determining seed quality. Moisture content, temperature, and relative humidity can influence the seed germination rate. The relative humidity has a significant impact on seed moisture content. Between the seeds and the surrounding environment, an equilibrium is always sought. As a result, when there is a greater relative humidity in the environment, the seed surface absorbs moisture from the air, increasing the crop's water activity. The rate of seed respiration increases as temperature and relative humidity during storage increase. As a result of the heat generated during respiration, the grain temperature rises, making it ideal for microbial infestation (Arief et al. 2020). The seed temperature further increases due to mould respiration. Microflora attack on the seed's germ produces a loss of dry mass and accelerates the seed's degradation (Sun et al., 2014). Canola with 45% (high) and 42% (low) oil content and initial moisture content of 8, 10, 12, and 14% was kept at 10, 20, 30, and 40 °C by (Sun et al., 2014). When compared to the lower oil content variety of canola, the rate of decrease in germination was significantly greater in the high oil content variety. After two weeks, seeds with a high moisture content of 12 and 14% at a temperature of 40 °C showed a decrease of 0-3 % in germination rate. Karunakaran et al. (2001) investigated the germination of wheat with five moisture levels of 15, 16 17, 18 and 18% (wet basis) held at different temperature regimes. They observed no differences in germination of wheat with 15 and 16% moisture content maintained at 25 °C for 70 days.

However, germination rate significantly reduced as storage duration increased in wheat with moisture content of 17, 18, and 19 %. Sathya et al. (2009) stored canola with four different moisture contents (7.5, 10.0, 12.5, and 15.0 %, wet basis) at 10, 20, 30, and 40 °C for 16 weeks to observe the quality changes (in environmental chambers with a 50 % RH). They reported that after 16 weeks of storage at 10 °C, germination rate of dry canola (7.5 and 10 % moisture content) was maintained over 80%, whereas canola possessing 7.5 % moisture content showed a germination rate of more than 80% after 16 weeks of storage at 20 °C. After three weeks of storage at 10 °C, germination rate of canola at 12.5 and 15.0 % MC, dropped below 80 %. Germination rate decreased below 80% in all the samples after 4 weeks of storage at high temperatures (30 and 40 °C). Rajaramanna et al. (2010) stored rye with moisture contents of 10.0, 12.5, 15.0, and 17.5% (maintained moisture contents after 16 weeks of storage) and compared the findings to those of Sathya et al. (2009) which reported that moisture content of rye significantly reduced with increase in storage period. Regardless of storage moisture content, rye kept at a low temperature (10 °C) maintained germination above 80% throughout the storage duration (16 weeks) in both trials. However, when kept at 20, 30, and 40 °C, germination of high moisture rye depicted a significant decrease as storage period increased.

2.4.5. Visible mould growth

The appearance of stored seeds is influenced by moulds that develop on it. The mould development on seed cause spoilage and value losses such as: 1) loss of viability; 2) discolouration of the entire seed or a portion of it; 3) loss or alteration of seed dry material; 4) selective destruction of specific seed tissues, such as the embryo of cereal seeds; 5) formation of unpleasant smells; 6) generation of toxic compounds that may affect human and livestock; and 7) "spontaneous" combustion on rare instances. *Aspergillus glaucus* impacted flaxseed mostly at 30 °C and 70% RH, while *Penicillium* spp. affected seeds at temperatures between 10 and 20 °C, according to White and Jayas (1991). According to research done by Sinha and Wallace (1977), *Penicillium, Cladosporium*, and *Aspergillus* species are mostly responsible for rapeseed (canola) rotting.

2.5. Storage period and safe storage guidelines

Seed storability is defined as the ability of seeds to remain viable throughout storage (Kandil et al. 2013). Temperature, seed moisture content, relative humidity, and storage period are all factors that affect the quality of stored grain. High temperature and MC are regarded to be undesirable storage conditions since they can cause changes in moisture content and biochemical processes in seeds (Strenske et al. 2017). Hence, it is ineffective to determine the feasible storage period without these factors (Singh et al. 2017). Storage guidelines have been developed for canola (Gunasekaran, 2006), wheat (Nithya et al., 2011), and other crops. However, few studies have been conducted on safe storage of flaxseed. To provide the market with high quality flaxseed, optimum pre-harvest practices, post-harvest management and storage conditions must be established. At present, a knowledge gap regarding the storability of flaxseed at different temperatures and moisture contents exists which need to be addressed.

2.6. Non-destructive analysis

From the previous section it can be established that the quality of seeds depends on its storage environment, moisture content and storage period. The change in these variables influences the seed quality hence affecting the end product use in the industry. Flaxseed is delivered to elevators and industries in the same way that other grains are, by rail cars, trucks, and ships. During transportation, the temperature and relative humidity of the seeds change, altering the moisture content of the oilseed. Before being processed by the industry, oilseeds can be kept whole and undamaged for a long time. The reduction in oil quality can be significantly accelerated if seeds are mechanically or frost-damaged, or if they become wet during harvest, handling, and storage owing to increased MC. Oilseeds' MC needs to be reduced to minimise degradation during storage, improve downstream processing efficiency, and allow for effective hull removal prior to oil extraction. To commence unit operations for oil extraction, the initial MC must be determined, and the flaxseed must be classified based on storage conditions. Using non-destructive analysis, systematic research may be used to assess flaxseed based on storage conditions and duration. This study uses non-destructive techniques, such as hyperspectral imaging and spectral profiles, to examine stored flaxseed.

2.6.1. Hyperspectral imaging

The electromagnetic spectrum includes photon energies for a range of electromagnetic radiations such as ultraviolet rays, x-rays, microwaves, radio waves, gamma rays, visible light, and infrared rays. They are divided into two categories: low frequency (long wavelength) and high frequency (short wavelength). When photons interact with matter, a spectrum is generated, and spectroscopy is the study of this interaction of light with matter. The visible range of light (400 to 740 nm) is used to sort food and agricultural commodities based on visual appearance (colour, morphology, or texture). The short wave near infrared (SWIR) range (900 to 2500 nm) is widely used to decipher the composition of organic materials, such as agricultural products.

Hyperspectral imaging (HSI) is a non-invasive, powerful, fast, accurate, and reliable approach for analysing biological materials. It uses a combination of imaging and spectroscopic techniques to analyse the nutritional profile and biochemical distributions in biological samples. It also includes details on the sort of chemical compounds contained in a sample's spectral dimension (Elmasry et al. 2012). Hyperspectral imaging is based on photon interactions with food at the molecular level. The sample's physical and chemical information is recorded in the form of a hypercube, a complex data unit. Hypercubes can provide both qualitative (e.g., disease infection) and quantitative (e.g., protein content) data. Area scan (staring imaging method), point scan (whiskbroom method), and line scan (push broom method) approaches can be used to obtain hypercubes.

Image acquisition in area scanning mode is done wavelength by wavelength within a fixed field of view. Spectra are obtained at points by moving the sample in point scanning mode. By collecting spectral data for a single spatial line, the line-scan or push broom approach allows for progressive hypercube construction. With the assistance of a transmissive or reflective diffraction grating or prism techniques, it creates a wavelength dispersive system. The samples can be physically relocated, or the beam and detector can be moved to the desired location.

Exploratory data analysis can be used to extract qualitative and quantitative information from hypercubes. Due to the large data dimension of hyperspectral images, it is critical to present the data efficiently by eliminating the extraneous data. To analyse data covariance structure, multivariate analysis employs a data dimension reduction approach. Principal component analysis

(PCA) is a data transformation technique that transforms a dataset into a linear combination of variables in smaller dimensions that are not associated with one another. It is the first stage in reducing a large amount of data and identifying key wavelengths. It is commonly used for data dimensionality reduction and outlier detection. PCA converts the original dataset into principal components using orthogonal linear transformation. Partial Least Square Discriminant Analysis (PLS-DA) is a supervised classification model that employs orthogonal latent variables (a set of new variables). The covariance between response variables and the design matrix is used to find latent variables. PLS-DA is a robust approach that combines the regression model (PLS) with the classification model, discriminant analysis (DA), to assign a dataset to one of the previously defined categories.

2.6.2. Application of hyperspectral imaging in grains and seeds

Hyperspectral imaging has been used by Williams et al. (2009) and Manley et al. (2009) to identify the firm and soft floury parts of the endosperm of maize kernels. Williams et al., 2009 used the wavelength ranges 960-1662 and 1000-2498 nm, while Manley et al., 2009 investigated the texture using the 1000-2498 nm wavelength. Furthermore, Sendin et al., (2018) used a 950-1700 nm wavelength to assess the chemical composition of maize, particularly the oil and oleic acid concentration. Xie et al. (2014), used wavelengths between 974 and 1734 nm to assess the oil content in sesame seeds in a similar study. This effort was successful, with a 96% accuracy. Wheat classification is one of the areas where the possibility of employing HSI technology has been explored and demonstrated to be feasible. Mahesh et al. (2008) investigated this and developed categorizations that were 90% accurate in classifying Canada Western Red Winter, Canada Prairie Spring Red, and Canada Western Soft White Spring wheat classes. Singh et al. (2010) established a technique that uses 1000-1700nm NIR HSI to help separate healthy wheat kernels from diseased ones, with an accuracy of 85-100 percent. Singh et al. (2010) used shortwave NIR HSI spanning from 700 to 1000 nm to distinguish between midge-damaged and healthy wheat kernels and found an accuracy of 95-99 percent. At 400-2500 nm, the amount of aflatoxin B1 in corn and barley was measured (Wang et al. 2015). The classification accuracy of infected kernels while operating at wavelengths of 400-1000 and 1100-1700 nm was 97 and 98%, respectively, (Kandpal et al. 2014, Wang et al., 2015). To identify different fungal-infected wheat kernels, NIR HSI of 1000-

1600 nm was used by Siche et al. (2016). In the same study a detection accuracy of 93, 99, and 87% was achieved for *A. niger*, *Penicillium* spp., and *A. glaucus*, respectively. A similar study was conducted by Delwiche et al. (2011) which used wavelengths of 1000-1700 nm (NIR) and 400-1000 nm (Visible range) to detect *Fusarium* in wheat kernels with an average accuracy of 95% but the accuracy reduced with decrease in the perceptibility of visual contrast between the wheat kernels. Various studies have investigated the corn quality, including the identification of *Fusarium verticillioides* infestations using images captured at wavelengths of 1000-2498 nm. There were also prominent peaks at 1900 and 2136 nm, which corresponded to variations in starch and protein composition. With time as the Y variable, partial least squares (PLS) regression models were developed. The coefficient of determination was determined to be 0.98 which established the feasibility to predict the degree of infestation (Williams et al. 2012). Siripatrawan and Makino (2015) studied the growth of *Aspergillus oryzae* in brown rice, using a wavelength of 400-1000 nm. Even though there were no symptoms in the kernels, detection was achievable, effective, and rapid, and the R^2 value was determined to be 0.97. Hyperspectral imaging has been effective in identifying the chemical compositions and infestations of cereals, legumes, and oilseeds, but no study has been done on flaxseed until now. The development of a non-destructive technology to determine the physical, chemical, and biological changes in flax during the storage period is necessary.

2.7. Research gap

To summarize, storage condition and storage time affects the chemical composition and viability of the seeds. Only two research studies have reported safe storage guidelines for flaxseed varieties commonly grown in North America (White, Mills, et al., 1999, White and Jayas, 1991). Over the last two decades, plant breeding has resulted in newer flax varieties with higher yield and disease resistant and suit in the North American Prairie climate (Flax Council of Canada, 2016). This research gap has also impacted the producers of flax and is identified as an area of high research priority by commodity organizations such as SaskFlax. There seem to be no studies to the best of our knowledge where non-destructive analysis techniques like hyperspectral imaging and spectroscopy has been used to study the effects of storage parameters on seed quality and classify it accordingly. Therefore, the objective of this study was formulated to develop safe storage

guidelines for Prairie grown flaxseed and classify the seeds based on storage conditions and storage period using non-destructive analysis.

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Chapter 3. Development of Safe Storage Guidelines for Prairie-grown Flax

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3.1. Abstract

Safe storage guidelines are essential to ensure that harvested commodities maintain the highest levels of quality during the varying environmental conditions through storage. These guidelines serve as effective tools for farmers to schedule appropriate post-harvest operations to prevent quality and quantity losses. The objective of this study was to develop safe storage guidelines for flax by investigating the effect of storage variables (storage temperature, moisture content, and storage period) on the seed deterioration parameters. The seed deterioration parameters include: germination, free fatty acid value (FAV), visible mould, protein content and moisture content (MC). To this end, flaxseeds were conditioned at 7, 8, 9, and 13% moisture content and stored at 10, 20, and 30 °C for a period of 16 weeks. Storage variables depicted statistically significant ($P = 0.05$) effect on the germination rate and FAV. Visible mould was apparent in the 4th and 3rd weeks for flaxseed with high moisture content (13%) stored at 20 and 30 °C. The protein content of flaxseed stored at different relative humidity conditions did not depict any statistically significant changes over the entire storage period, but the significant changes were observed over the various temperature ranges. It was concluded that safe storage of flaxseed can be ensured at 10 and 20 °C for more than 16 weeks if the moisture contents are maintained at 7, 8, and 9%. Under rare scenarios, if the crop is to be stored at a high moisture content (~13%) and at temperatures of at least 20 °C, it must be dried to lower moisture levels to within 3 weeks of storage. When storing flaxseed for an extended period of time, it is recommended to keep the temperature below 20 °C to ensure high quality and to maintain seed viability.

Keywords: Flaxseed, relative humidity, temperature, moisture content, germination

3.2. Highlights

- Safe storage guidelines for Prairie-grown flaxseed were established

- Flaxseed stored at high temperature and moisture content are prone to quicker deterioration
- Long term safe storage of flax can be ensured if the moisture level is kept between 7 to 9%

3.3. Introduction

Canada has been the leading producer of flax (*Linum usitassimum*) worldwide since 1994. Statistics Canada reported that in 2019, 483.2 thousand tonnes of flaxseed were produced utilizing an area of 376.0 thousand hectares. Flaxseed possesses a rich nutritional profile comprising approximately 28-30% protein, 35% fibre, and 34-45% oil (from which 35-45% is alpha-linolenic acid (ALA)), (Mishra & Awasthi, 2021) making it a crop of high economic value. In 2014, Health Canada approved flax as a functional food linking the consumption of whole flaxseed to lower blood cholesterol levels (Goyal et al. 2014). Flaxseed proves to be the best source of omega-3 fatty acids for the vegetarian and vegan consumers. Edible flax is consumed both in as raw whole seed and is used in other foods, such as salads, bread, muffins, spaghetti, and energy snack bars (Kajla et al. 2015).

Flaxseed is harvested and stored at a moisture content of <10% for long-term storage (Ehrensing 2008). The quality of the stored seeds can be affected by biotic and abiotic parameters. The biotic factors comprise of insects, microorganisms, mites, and rodents, whereas abiotic factors include moisture content (MC) of the seed, temperature, seed type, and storage structure (Nithya et al. 2011). These factors can significantly influence the economic value of the crop during storage as they directly contribute to quality degradation.

The key determinants of the grain crop storage ecosystem are seed MC and temperature, which can be manipulated to ensure optimum storage conditions necessary to ensure quality and monetary value of the crop (Rani et al., 2013). The quality parameters commonly assessed are germination, protein content, free fatty acid value (FAV), fungal growth, nutritive variations, moisture adsorption, and seed coat color (Jones, 1999; Muir, 2001). During bulk storage of crops, high moisture pockets can cause increased water activity, which in turn results in non-enzymatic browning, enzyme activity, lipid oxidation, and microbial growth. The deterioration of stored commodities cause decrease in seed germination and increase in FAV. Similar trends have been observed in wheat (Nithya et al., 2011), rye, and canola (Sathya et al., 2008, 2009).

Safe storage guidelines are imperative to ensue crop safety and are experimentally determined within the expected ranges of moisture levels and temperatures during storage. These guidelines can assist farmers in scheduling different post-harvest operations such as drying, cooling, turning, etc. to avoid crop spoilage (Sathya et al., 2009). Considering each crop variety interacts differently with the biotic and abiotic elements of its storage environment, such guidelines are essential for

the post-harvest management of every commercially grown commodity. Moreover, as newly bred varieties replace older varieties, safe storage guidelines need to be updated periodically. Safe storage guidelines have been developed for canola (Sun et al. 2014), wheat (Nithya et al., 2011), red lentils (Cenkowski et al. 2015), soybeans (Diaz-Contreras et al. 2021) etc. However, according to Flax Council of Canada, there exists a knowledge-void when it comes to safe storage of flax, especially given the resurgence of the crop's popularity and economic relevance. The only two research studies done on flaxseed varieties in North America are grossly outdated (White, Mills, et al., 1999, White & Jayas, 1991).

Over the last two decades, plant breeding has resulted in newer flax varieties more suited for cultivation in the North American Prairie climate. This gap in knowledge has adversely impacted the producers of flax and is identified as an area of high research priority by commodity organizations. Therefore, the objective of this study was formulated to develop safe storage guidelines for Canadian prairie-grown flaxseed.

3.4. Materials and methods

3.4.1 Selection of storage temperature and moisture regimes

The optimum storage temperature is determined considering factors such as harvesting period, minimum and maximum environmental temperature, and the temperature and moisture of the seed as it goes in storage. According to Rani et al. (2013), the average 24 h daily temperature of the Canadian Prairies is around 25°C during flaxseed harvesting season. Microbial stability of the stored seeds can be ensured if the storage temperature is ≤10°C. Taking this into consideration, the storage temperatures selected for developing safe storage guidelines were chosen as 10, 20 and 30°C (±2) and the RH conditions were decided to be maintained at 55, 65, 75 and 94% to imitate the variations in the environmental conditions of the Prairies. The desired RH was achieved by using four saturated salt solutions: $Mg(NO_3)_2$, $NaNO_2$, NaCl, and KNO_3 to produce 54, 65, 75 and 94% RH, respectively. The RH inside the environmental chambers were maintained from 60 to 70% throughout the study.

3.4.2. Sample preparation

Flaxseed samples were obtained from a local producer in Manitoba at an initial MC (wet basis) of 7.5-8%. Conditioning of the grain samples was done to achieve the required moisture contents (7,

8, 9 and 13%) by adding calculated quantities of distilled water into a batch tumbler using the Equation (1).

$$Water\ added = Weight\ of\ grain \times ((Mf - Mi)/(1 - Mf)) \times 100 \qquad \text{(Eq: 1)}$$

Mf = MC to be achieved Mi = Initial MC

These MC levels were selected to represent the different Canadian harvest seasons (i.e., dry, intermediate, and wet). The seed was conditioned for 2 h at 36 rpm in a metal-lined batch tumbler (24 × 24 × 51 cm). Every 30 mins the lid was removed, and clumps of seeds were broken to ensure minimum breakage and uniform moisture penetration. The seeds were then placed in sealed double plastic bags leaving sufficient headspace to facilitate further moisture equilibration. The bags were kept at room temperature for 6 h. Flaxseed in the bags was shuffled at regular intervals every 3 hours after which the bags were placed in an environmental chamber (Conviron CMP3244, Controlled Environments Ltd., Winnipeg, MB) at 5°C for 72 h. Final moisture contents of the samples were verified by the hot air oven method (ANSI/ASABE 2012). The samples were kept in polythene bags at 5°C until used for the experiments.

3.4.3. Experimental setup

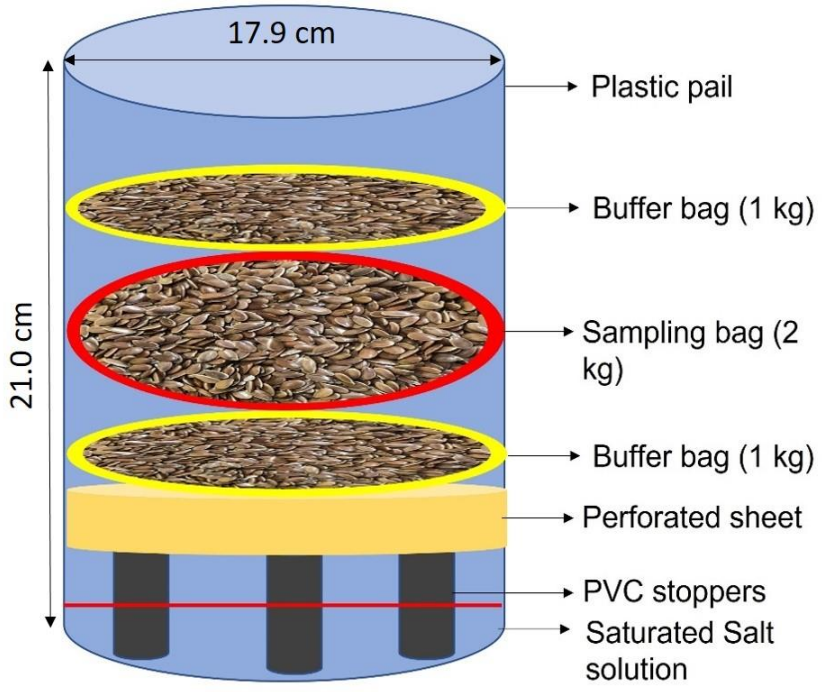

Fig. 3.1. Schematic model of the experimental arrangement of flax samples in 5 L pail.

For the storage experiment, 2 kg of conditioned flaxseed were kept inside polyester mesh bags with a mesh size of <1.168 mm as the main sample. To prevent moisture loss from the main sample due to regular opening of the pale during to sample collection, 2 kg of buffer sample were placed above (1 kg) and below (1 kg) the sampling bag (Nithya et al., 2011). These bags were kept in a cylindrical plastic pail (17.9 cm diameter and 21 cm high) of 5 L capacity. The sampling and the buffer bags were kept on a perforated plastic sheet above the saturated salt solutions which was supported by three PVC pipes (about 8 cm in diameter and 6 cm high) as shown in Fig. 3.1. Half the height of the PVC pipes was submerged in saturated salt solutions. The plastic pail was loosely covered with a lid for air exchange. It was assumed that shuffling the seeds in the sampling bags and uncovering the pails on a weekly basis minimized anaerobiosis and the accumulation of gaseous metabolites from seeds and fungi (Mills et al. 1996). There were three replicates for each temperature and moisture combination. A total of 12 pails were stored in each of the three different environmental chambers which were set at 10, 20, and 30 °C (± 2 °C). Temper and relative humidity sensor (HOBOs) were placed in every pail to ensure the required temperature and Rh is attained. The grain in the sampling bag was mixed thoroughly and taken for analysis at weekly intervals for 16 weeks.

3.4.4. Qualitative parameters

About 100 g of seeds from each sampling bag were acquired on a weekly basis to determine the MC, FAV, seed germination, visible mold, and protein content. The germination rates were assessed by placing 25 seeds on Whatman no. 3 filter paper in a 9 cm diameter Petri-dish saturated with 5.5 mL of distilled water (Wallace and Sinha 1962). Sample MC was determined on wet basis, using the ASAE Standard S352.1 by drying whole seeds in a convection oven 5-10 g of whole flaxseed at 103°C (± 2°C) for 4 h using duplicate subsamples. The FAV of stored flaxseed was determined using fat extractors (Goldfisch Fat Extractor, Laboratory Construction Co, Kansas City, MO). The calculated FAV was then expressed as mg KOH/100 g of dry grain (Karunakaran 1999). The protein content was analysed using a LECO apparatus (LECO, Empowering Results, MI, USA). Appearance of visible mould was monitored by visually inspecting the samples with naked eyes.

3.4.5. Statistical analysis

Analysis of variance (ANOVA) was done using a three-way factorial design model (4 moisture contents × 3 storage temperatures × 16 weeks) to study the effect of all these three independent variables on the dependent variables (seed germination, FAV, protein content, and moisture content). The assumptions of ANOVA were tested for homogeneity and normal distribution. The assumption of homogeneity of variance was tested using Levene's test. General linear model procedure with multivariate analysis were conducted using SPSS software (Version 25). Pairwise comparisons between the quantitative variables coming from three or more independent groups, and to analyze the significant changes in germination, protein content, MC and FAV during storage period was done using least significant difference (LSD) and Tukey's tests. The differences within each level under each variable were tested at a 95% confidence interval.

3.5. Results and discussion

3.5.1 Free fatty acid value

An increase in FAV was observed with increase in moisture content, relative humidity, and storage period. After the initial moisture conditioning, a difference in the FAV was observed for each conditioned batch of flaxseed. The average initial values of FAV were 7.38 ± 0.65, 8.07 ± 0.74, 9.15 ± 0.70, and 14.50 ± 2.98 mg KOH/100 g of dry flaxseed for 7, 8, 9, and 13% initial moisture content, respectively. The maximum increase in FAV was observed in samples with the highest moisture content of 13% probably due to rapid hydrolysis by fungal enzymes (White et al. 1999). Flaxseeds stored at the initial moisture content of 13% showed almost a fivefold increase in FAV at 20 and 30 °C as shown in Fig. 3.2 b and c, respectively. A significance difference was observed between the FAV and storage temperature, initial moisture content, and storage period of flaxseed as per the multivariate analysis, LSD, and Tukey tests (Table 3.1).

Table 3.1. ANOVA table for FAV of stored flaxseed.

Independent variable	Dependent variable	Df	F	Sig.
Temperature	FAV	2	6355.332	0.000
ERH		3	13118.184	0.000
Storage Period		4	1984.715	0.000
Temperature * ERH		6	1880.448	0.000
Temperature * Storage Period		8	368.586	0.000
ERH * Storage Period		12	1035.737	0.000
Temperature * ERH * Storage Period		20	239.327	0.000

A previous study on pinto beans observed a three-fold increase from the initial FAV (7.9 mg KOH/100 g of dry beans) for samples that were stored at high moisture content and high temperature (Rani et al. 2013). Gunasekaran (2006) reported FAV of 590 mg KOH/100 g of dry canola stored at high moisture content (15%) and high temperature (30 °C) at the 4^{th} week of storage. This shows a 26-fold increase from the FAV at the beginning of the storage period of canola samples (22.02 mg KOH/100 g of dry canola). Increased temperatures accelerate the oxidation of lipids by lipolytic enzymes. Similar trends were also observed for rye and canola (Rajarammanna et al., 2010; Sun et al., 2014). In a study conducted on Cannabis sativa L., relative humidity was found to be the most significant factor that influenced FAV (Jian et al. 2019). High relative humidity increases the activity of enzymes found in seeds and metabolic activity in moulds increasing its proliferation, favouring the increase of free fatty acid content in oils (Jian et al. 2018 ; Nithya et al. 2011; Esther et al. 2015). It was observed that the samples stored at lower relative humidity (54 and 65%) had the lowest FAV. These findings complemented those of Chidananda et al. (2014)'s chickpea research.

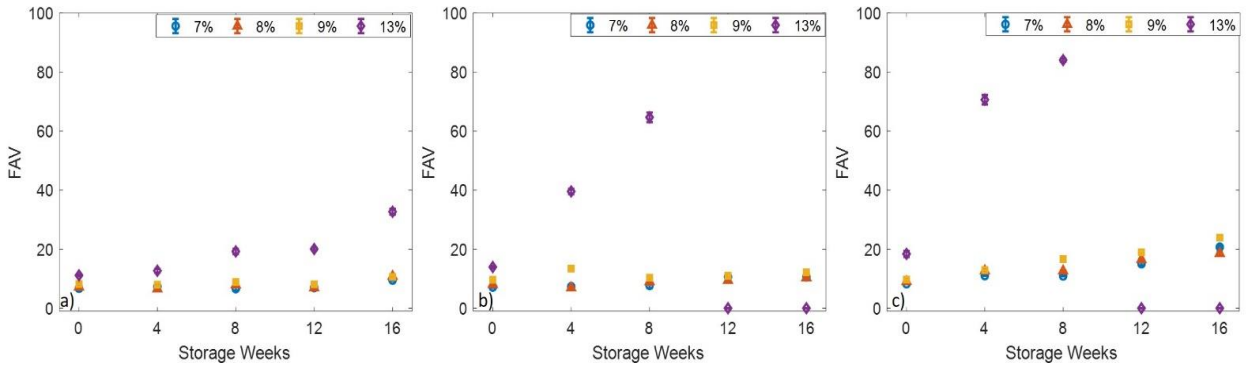

Fig. 3.2. FAV (mg of KOH/100 g of dry flaxseed) of stored flaxseed over 16 weeks of storage period at: a) 10 °C, b) 20 °C, and c) 30 °C. Error bars represent standard deviation.

3.5.2. Seed Germination rate

Seed germination is often used as a criterion for determining the quality of stored grains. Reduced germination in storage could be an indicator of fungi infestation. The influence of storage factors (initial MC, temperature, and storage period) on germination rate of the samples (Total number of samples = n = 36 every week) was found to be significant ($P < 0.05$) as observed in Table 3.2. The initial germination of the seeds was 98.6 ± 2%. The germination of samples with 7, 8, and 9% moisture content at 10 °C was observed to be 90.6 ± 2.3, 92 ± 0.01, and 85.3 ± 2.3%, respectively for the 16 weeks of storage as shown in Fig. 3.3a. After the 15th week of storage, the germination of seeds having 13% initial moisture content at 10 °C dropped down to 73.31 ± 2.3%. At 20 °C, the germination of flaxseed with 7, 8, and 9% MC was observed to be >80% for the entire period of storage. But the germination of samples stored at 20 °C at 94% Rh was observed to be below 70% after 8 weeks of storage (Fig. 3.3b). Fig. 3.3 c shows a rapid decrease in germination when stored at 30 °C. The seed germination at 7 and 8% moisture content was observed to be 81.3 ± 2.3% and 74.6 ± 2.3%, respectively. The germination rate of samples stored at 9 and 13% MC dropped below 70% after the 15th and 6th week of storage, respectively.

Table 3.2. ANOVA table for germination of stored flaxseed.

Independent variable	Dependent variable	Df	F	Sig.
Temperature	Germination	2	229.345	0.000
ERH		3	303.749	0.000
Storage Period		4	340.757	0.000
Temperature * ERH		6	39.807	0.000
Temperature * Storage Period		8	46.432	0.000
ERH * Storage Period		12	56.606	0.000
Temperature * ERH * Storage Period		20	13.111	0.000

The germination rate is inversely related to the storage temperature and storage period as reported in previous studies including studies on wheat (Karunakaran et al., 2001; Nithya et al., 2011), canola, rye, black grams, and pinto beans (Sathya et al., 2008, 2009; Rajarammanna et al., 2010; Esther et al. 2015; Rani et al. 2013). Crops harvested under rainy conditions (high relative humidity and high MC) showed lower germination rates. Jian et al., (2019) reported that seed germination could be predicted by multiple regression models based on storage time, temperature, FAV, and moisture content for barley, oats, and hemp. According to the same study, relative humidity, along with storage period, was one of the key factors influencing germination. Germination of samples held at % RH (15.5 % MC) and 35°C was reduced by more than half in the first week in research on industrial hemp. This finding was in line with wheat (Al-Yahya, 2001) and soybean literature (Kandil et al., 2013). On the contrary, the correlation was demonstrated to be inconsistent by Sun et al. (2014) for canola. Nithya et al. (2011) observed a significant effect of increasing temperature and relative humidity on wheat. Moreover, crops stored at > 9% MC were also prone to spoilage if stored above 20 °C.

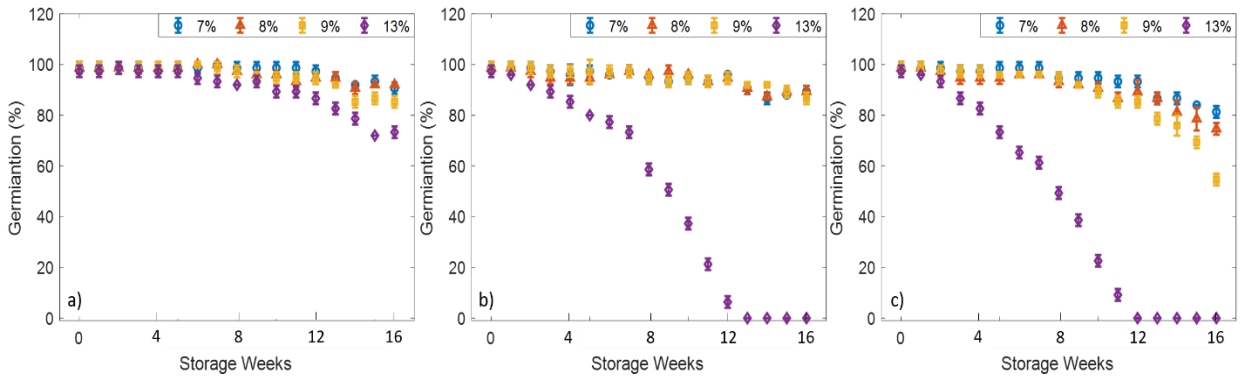

Fig. 3.3. Germination rate of stored flaxseed over 16 weeks of storage period at: a) 10 °C, b) 20 °C, and c) 30 °C. Error bars represent standard deviation.

3.5.3. Moisture content

Moisture content is a critical factor to consider when storing crops for long periods of time. It changes the seed characteristics and creates an environment that is conducive to the growth of insects and microbes. The four levels of initial moisture content (7, 8, 9 and 13%) were maintained at the desired levels by controlling the equilibrium relative humidity (ERH) around the flaxseed samples using different saturated salt solution along with buffer bags throughout the storage period. An increase in moisture during the overall storage period at all the temperatures was observed in samples at 7, 8, and 9% MC. For samples stored at 10 °C, the moisture content was statistically significant as compared to those stored at 20 and 30 °C (Fig. 3.4a). During storage, the moisture content of seeds did not show any significant difference between 0^{th}, 4^{th} and 16^{th} week (day 1, day 28 and day 112). Significant differences among the moisture contents based on the relative humidity (Table 3.3). With the values of multivariate analysis shown in Table 3.3, it is evident that there is no significant change in MC over the range of temperatures. It was also confirmed by LSD and Tukey's tests that there was no significant difference in moisture content between samples stored at 20 and 30 °C through the entire storage period.

Table 3.3. ANOVA table for MC of stored flaxseed.

Independent variable	Dependent variable	Df	F	Sig.
Temperature	Moisture content	2	1.512	0.225
ERH		3	4162.329	0.000
Storage Period		4	76.823	0.000
Temperature * ERH		6	16.264	0.000
Temperature * Storage Period		8	1.737	0.097
ERH * Storage Period		12	24.432	0.000
Temperature * ERH * Storage Period		20	3.369	0.000

Sravanthi et al., (2013), Nithya et al., (2011), (Ziegler et al. 2016) and Rani et al., (2013) also noticed a rise in the initial moisture content of red lentils, durum wheat, soybeans and pinto beans stored at similar temperatures. From Fig. 4b and c, a decrease of moisture content was observed at 20 and 30°C in flaxseed stored at high moisture contents (i.e., 13%). Majid et al., (2014) discovered a similar rise in moisture content because of the water activity of chickpeas. The findings were consistent with those of black gram samples, where changes in storage and ambient temperatures caused a divergence from the equilibrium relative humidity, leading to a change in the moisture content of the sample (Esther et al. 2015).

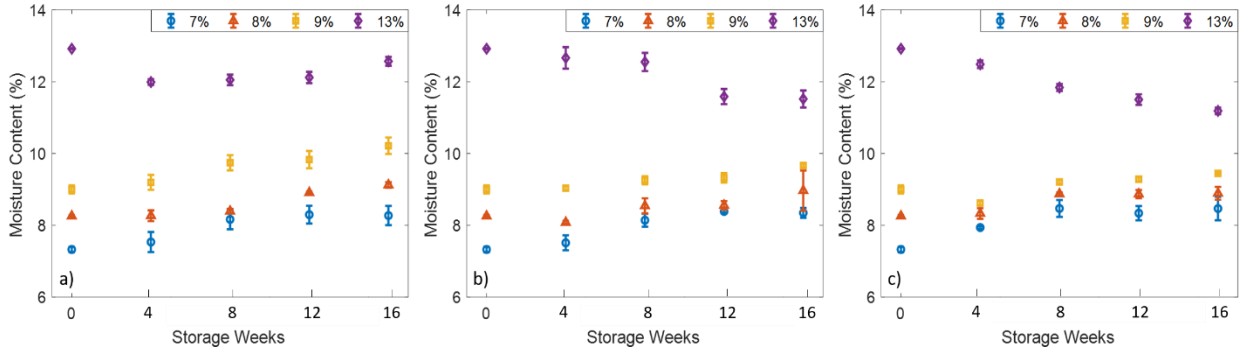

Fig. 3.4. Moisture content (% wet basis) of stored flaxseed over 16 weeks of storage period at: a) 10 °C, b) 20 °C, and c) 30 °C. Error bars represent standard deviation.

3.5.4. Visible mould

The appearance of visible mould was observed in the 4th and 3rd week for the seeds stored at high moisture content (13%), and high temperature (20 and 30°C), respectively (Table 3.4). During the 14th week, presence of mould was observed in flaxseed at 9% moisture content stored at 30°C. For samples stored at 10 °C, visible mould was observed at 13% moisture content in the 16th week of storage. Similar trends were observed by Gunasekaran (2006) in rye and canola. Higher moisture content samples showed the appearance of visible mould regardless of the storage temperature. Rani et al., (2013) observed the initial appearance of mould mainly near the hilum and cracks around the seed coat of pinto beans. The appearance of visible mould in most stored seeds is considered as the first step towards seed deterioration. Except for canola as reported by Sun et al., (2014) where the germination was observed above 80% even on the onset of visible mould when stored at 12% MC at 20 °C.

Table 3.4. First appearance of visible mould and germination rate (%) of flax during storage ("-" indicated no appearance of visible mould).

M.C (wet basis %)		7	8	9	13
Storage temperature (°C)	Replicate	Week, Germination %	Week, Germination %	Week, Germination %	Week, Germination %
10	1	-	-	-	16,73
	2	-	-	-	-
	3	-	-	-	-
20	1	-	-	-	4, 84
	2	-	-	-	4, 88
	3	-	-	-	4, 84
30	1	-	-	-	3, 88
	2	-	-	-	3, 88
	3	-	-	14, 72	3, 84

3.5.5. Protein content

Initial protein content of the conditioned flaxseed was determined to be 21.44 ± 0.3%, 21.48 ± 0.14%, 21.42 ± 0.15%, and 21.35 ± 0.2% for 7, 8, 9, and 13% initial moisture content, respectively. For all levels of relative humidity conditions, the crude protein content did not show any statistically significant change over the storage period. Significant changes were observed in samples over the temperature ranges and the overall storage period (Table 3.5). The effect of relative humidity on the stored sample was not significant over the storage period. The result was consistent with Majid et al. (2014) where protein content of chickpeas changed insignificantly with the combined as well as independent effect of water activity and storage time. Sravanthi et al., (2013) investigated the effect of storage conditions on protein content of red lentils and observed no significant differences during the 16-week storage period. Rani et al. (2013) discovered a statistical difference in protein content and moisture, as well as an inversely proportional relationship between the two variables.

Table 3.5. ANOVA table for protein content of stored flaxseed.

Independent variable	Dependent variable	Df	F	Sig.
Temperature	Moisture content	2	7.139	0.001
ERH		3	.631	0.596
Storage Period		4	16.617	0.000
Temperature * ERH		6	1.170	0.327
Temperature * Storage Period		8	4.945	0.000
ERH * Storage Period		12	.657	0.788
Temperature * ERH * Storage Period		20	.837	0.665

3.6. Estimated Storage life

Fig. 3.5 depicts a safe storage guideline chart for flax storage periods at different temperatures and moisture levels based on seed germination and the first appearance of visible mould. The seeds may be safely stored at 7, 8, and 9 % moisture content at 10 and 20 °C for at least 16 weeks with no quality degradation. To ensure preservation of quality, flaxseed at 13% moisture content at 20 and 30 °C must be dried below 8 - 9% to approximately before 3 weeks within storage. Although

not recommended but in exceptional circumstances, if the crop is to be stored at 30 °C, it must be dried to 8% MC before 10 weeks within storage. When storing flax for an extended period of time, it is recommended to keep the temperature below 20 °C to ensure high quality and seed viability. The previously developed storage guidelines for flaxseed depicts spoilage of seeds stored between 6 - 13% MC when stored at 5–45 °C (https://www.grainscanada.gc.ca/en/grain-quality/manage/manage-storage-prevent-infestations/prevent-spoilage.html). The "no spoilage" zone marks safe storage for 5 months. Similar trend, depicting the spoilage of flaxseed was observed by both previously developed and currently developed storage chart (Fig 3.5), when stored at 9 and 13% MC at 20 and 30 °C. The previously developed chart showed spoilage of 8% MC seeds at 30 °C for storage period of 4-5 months whereas no spoilage was observed in current study for similar storage condition. No spoilage should occur according to both the charts for samples stored at MC < 7% when stored at maximum of 30 °C for 5 months of storage.

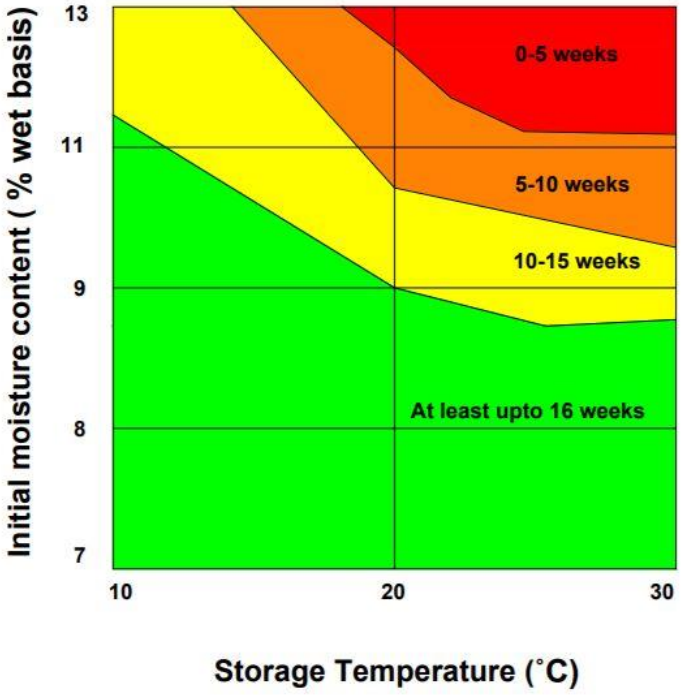

Fig. 3.5. Recommended safe storage guidelines chart for flaxseed starting from the week when placed in storage.

3.7. Conclusions

The moisture content, storage temperature, and storage period depicted statistically significant effect on the seed germination and FAV. The increase in storage variables (temperature, MC and storage period) was inversely proportional to flaxseed germination rate whereas a proportionality was depicted in case of FAV. Germination rate was maintained above 80% for samples with 7, 8, and 9% initial moisture content stored at 10 and 20 °C throughout the storage period. On the other hand, germination significantly decreased for high moisture content seeds (13%) when stored at 20 and 30 °C. Viability of flaxseed stored at 13% moisture was lost in the 12^{th} and 11^{th} weeks for samples stored at 20 and 30 °C, respectively. The germination rate and FAV were found to be inversely proportional to each other as the germination rate decreased the FAV increased. For samples at 20 and 30 °C and 13% MC, the visible mould was observed within the first 4 weeks of storage. It is recommended that for longer storage periods the flaxseed should be stored below 9% moisture content and 20 °C to maintain high seed quality.

3.8. Acknowledgements

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Chapter 4. NON-DESTRUCTIVE QUALITY MONITORING OF FLAXSEED DURING STORAGE

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4.1. Abstract

Fluctuating environmental conditions during storage and transportation significantly influence the quality parameters of flaxseed. Considering that over 80% of Canadian flaxseed is exported, the industry is in dire need of a rapid and reliable technique to assess its quality quickly and non-destructively at points of trade. This study was aimed at finding a method to classify of flaxseed based on different storage conditions followed by prediction of quality parameters during a storage period of 16 weeks. To this aim, principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA) and partial least squares regression (PLSR) models were developed in the Vis-NIR (450-1100 nm) and SWIR (1100-2500 nm) ranges. Flaxseed samples possessing initial moisture content (MC) of 7, 8, 9, and 13% were stored at 10, 20, and 30 °C under 54, 65, 75, and 94% relative humidity (RH) conditions, respectively. Unsupervised PCA models were used for initial data exploration. Supervised PLS-DA model for storage time-based classification yielded non-error rates of 77.6, 72.2, and 77.4 % in calibration, cross-validation, and external prediction, respectively. For the classification of flaxseed based on initial MC, the PLS-DA model yielded classification accuracies of 81.5, 72.8, and 87.5% in calibration, cross-validation, and external prediction, respectively. The PLSR prediction model for MC yielded a coefficient of determination (R_c^2) of 0.96, in cross-validation (R_{cv}^2) of 0.95 and in prediction (R_p^2) of 0.97 with root mean square error in calibration (RMSEC) of 0.34, RMSECV of 0.38, and RMSEP of 0.27. Free fatty acid value (FAV) was predicted with an R_p^2 of 0.76 and a RMSEP of

6.44. Hence, it was concluded that NIRS can be used by traders, food processors, and farmers as a reliable tool to non-destructively assess flaxseed quality for making logistical decisions.

Keywords: spectroscopy, multivariate data, classification, prediction, Near-infrared spectroscopy (NIR)

4.2. Highlights

- Wavelength range of 650-750 nm can be used to classify flaxseed based on storage time
- Peaks at 1400 nm and 1900 nm contributed towards amount of moisture in flaxseed and can be used in classification based on initial moisture content
- Moisture and fatty acid contents were successfully predicted in the SWIR range
- NIRS is a reliable tool for flaxseed quality monitoring during storage

4.3. Introduction

Canada is the largest producer of flaxseed in the world, accounting for approximately 80% of flax commerce. Flaxseed has sparked renewed interest in the field of nutritional research over the last two decades, due to the abundant presence of α-linolenic acid (ALA, omega-3 fatty acid), lignans, fiber, protein, and an array of antioxidants [1]. Its consumption is associated with reduced risks of cardiovascular disease, cancer, anti-inflammatory activity, and has laxative properties. Baked goods, dairy products, dry pasta products, and meat items often include flaxseed or its oil, highlighting its importance as a functional food ingredient [2].

The suitability of flaxseed for a specific end product depends upon factors such as fat and protein content, which can be significantly impacted by storage conditions. Since flaxseed retains high respiration rate up to 6 weeks after harvest, the metabolic activity continues even after harvest as the crop goes into storage. These biochemical processes break down the carbohydrates into water, heat, which increase the moisture content, thereby creating ideal conditions for seed spoilage by promoting microbial growth. It is important to note that deterioration of functional compounds could start well before any damage is visually apparent. Therefore, assessment of flaxseed's chemical composition to determine its safety and quality is a key requirement for the industry. Soxhlet extraction is a popular solvent extraction technique that is conventionally used for the determination of oil content in seeds [3]. Crude protein content, on the other hand, is typically assessed using the Kjeldahl and Dumas combustion methods [4]. The moisture content of grains and oilseeds is typically determined using oven drying method. It is important to note that all these analytical techniques require time, skilled labour, extensive sample preparation and, in most cases, use of environmentally hazardous chemical compounds. To this end, conventional approaches such as Soxhlet extraction, Kjeldahl and Dumas combustion, oven drying methods, gas chromatography (GC), and GC coupled with mass spectrometry (GC-MS) are utilized to determine

various quality attributes. Other more specialized methods for characterizing seed quality include moisture measurement using Terahertz spectroscopy and low resolution pulsed nuclear magnetic resolution [5; 6], GC, and GC-MS to analyze fatty acid composition [7].

Approximately 80% of Canadian flaxseed is shipped to China, USA, and Europe. The seeds are transported in the same manner as other grains, by railcars, trucks, and ships, and is received at the elevators. The temperature and relative humidity of the seeds change throughout transit, affecting the oilseeds moisture content and FAV. Moreover, these oilseeds could stay in storage for extended periods before being processed by the industry. If seeds are mechanically or frost-damaged, or if they gain moisture during harvest, handling, and storage, the decline in oil quality can significantly worsen due to increased moisture content (MC). The MC of oilseeds is frequently lowered to prevent storage deterioration, enhance downstream processing efficiency, and permit efficient hull removal prior to oil extraction. To begin unit operations for oil extraction, it is imperative that initial MC is rapidly assessed and the quality deterioration, if any, as a consequence of storage, determined non-destructively in real time.

To meet increasingly stringent regulations and higher consumer expectations, the modern food processing industry is transitioning towards rapid, environmentally sustainable, and cost-effective methods for oilseed quality estimation. An ideal non-destructive technique that has already established itself as an alternative to chemical analyses in the agri-food industry is near infrared spectroscopy (NIRS). Combined with multivariate data processing approaches, NIRS has been successfully used for compositional analyses of cereal grains [8], oilseeds [9], fruits and vegetables ([10]; [11]), and leguminous crops [12].

Studies have also been conducted to determine various aspects of flax quality using NIRS. Enakiev et al. (2018) and Huang & Yu, (2020) used NIRS in the wavelength ranges of 680-2500 nm and

1000-2500 nm, respectively for the determination of cellulose, hemicellulose, and lignin content in flax fiber [13; 14]. Both research studies concluded that NIRS is a reliable and rapid method for the determination of flax fiber composition. NIRS has also been utilized for the estimation of the limit of cleanliness of flax fiber to achieve high-value fibers for uses in textile [15]. It was concluded that determination of the extent of fiber cleanliness was possible using NIRS. Faughey & Sharma (2000) used NIRS to develop calibration models for predicting important fiber parameters [16]. They revealed that wavelength range of 1800-2498 nm played a significant role in modelling the difference in the fineness of flax fiber. On the other hand, the wavelength range of 1800-2498 nm was also effectively used to correlate the fiber strength with the spectral measurements. Additionally, mid- and near-infrared spectral ranges have been used for the prediction of linoleic acid in yellow and brown flaxseed [17]. The results indicated that the prediction models developed in the NIR spectral range for prediction of linoleic acid provided accurate results with an R^2 of 0.90 and standard error of prediction of 1.61. Furthermore, it was established that NIRS and mid-NIRS can effectively be used to predict linoleic acid in whole flaxseed and flax flours. Principal component analysis (PCA) can be used for the classification of different flax cultivars in the Vis-NIR range of 500-720 nm [18]. The first principal component covered 99.78% of the total variance in the data resulting in reliable classification in this wavelength range. Owing to the advantages of NIRS over tedious chemical analysis and the technique's success in estimating the various quality attributes of flaxseed, it was deemed as an ideal tool to study the quality changes that occur in flaxseed during storage. In the present study, flaxseed possessing four different initial moisture contents of 7, 8, 9, and 13% were stored at 10, 20 and 30°C under four different relative humidity conditions for 16 weeks and changes in its quality were monitored. Vis-NIR and SWIR wavelength ranges were explored for the prediction

of quality parameters (viz. moisture content, FAV, protein content, and germination) during storage. Classification of Prairie grown flaxseed samples was also done based on their storage conditions including storage period (16 weeks), storage temperature (10, 20, 30°C), relative humidity (54, 65, 75, 94%) and initial MC (7, 8, 9, 13%).

4.4. Materials and methods

4.4.1. Sample Preparation

Flaxseed samples were obtained from a local producer in Manitoba at an initial moisture content (wet basis) of 7.5-8.0%. To reach 7% MC the seeds were dried at room temperature (20 ± 2°C) for 48 hours. The seeds were conditioned to reach the remaining three different initial moisture contents (8, 9, and 13%) by adding a calculated quantity of distilled water into a metal-lined batch tumbler (24×24×51 cm) where they were turned intermittently until water was uniformly absorbed. After conditioning, the seeds were placed in sealed double plastic bags leaving sufficient air space to facilitate further equilibration of moisture. Final moisture contents of the flaxseed were verified using the oven drying method [19]. The bags were kept at room temperature for 6 h and were shaken at regular intervals of 3 hours followed by storage at 5°C for 72 h for equilibration of moisture throughout the seeds. To mimic storage conditions on the Canadian Prairies, a temperature range of 10, 20, and 30°C was selected for each of the environmental chambers (Conviron CMP3244, Controlled Environments Ltd., Winnipeg, MB, Canada). Four saturated salt solutions, namely $Mg(NO_3)_2$, $NaNO_2$, NaCl, and KNO_3, were used to attain approximately 54, 65, 75, and 94% RH in storage, respectively. Each storage chamber (10, 20, 30°C) contained 12 pails, 3 from each initial moisture content (7, 8, 9, 13%). 2kg of conditioned flaxseeds were placed inside the pails for sampling. The plastic pails were loosely covered with a lid for air exchange. Mixing of the seeds was done on a weekly basis to minimize anaerobiosis and the accumulation of gaseous metabolites from seeds and fungi [20]. The flaxseed in the mesh bags was mixed thoroughly and

taken for analysis every week over a period of 16 weeks. Three replications were done for each temperature and moisture combination.

4.4.2. Qualitative parameters

During the 16-week storage period, 100 g of flaxseed were acquired from each sampling bag on a weekly basis to determine seed germination, MC, FAV, protein content, and visible mould. Germination rate was assessed by placing 25 seeds on Whatman No. 3 filter paper in a 9 cm diameter Petri-dish saturated with 5.5 mL of distilled water [21]. American Society of Association Executives Standards (ASAE Standard S352.1) was used to determine MC of flaxseed on wet basis by drying 5-10 g of whole flaxseed in a convection oven set at 103°C (± 2°C) for 4 h using duplicate subsamples. The FAV of stored seeds was determined using fat extractors (Goldfisch Fat Extractor, Laboratory Construction Co, Missouri, USA). The calculated FAV was then expressed as mg KOH/100 g of dry grain [22]. LECO apparatus (LECO, Empowering Results, St. Joseph, MI) was utilized to determine the protein content. Flaxseed were visually inspected to detect the appearance of visible mould.

4.4.3. Hyperspectral imaging systems

Vis-NIR and SWIR line scanning hyperspectral imaging systems (SPECIM Spectral Imaging Ltd., Oulu, Finland) were used to acquire hyperspectral images (HSI) of the flaxseed samples. The Vis-NIR system possessed a charge coupled device (CCD) camera with a sensor array of 1024 × 896 pixels. The camera had a spectrograph (SPECIM V10E) that operated in the wavelength range of 397.66-1003.81 nm along with a spectral resolution of 2.6 nm and a focusing lens (SPECIM OLET 15). Illumination was provided by two 150 W tungsten lamps (3900-ER, Illumination Technology, Inc., New York, USA) positioned at an angle of 45° from the sample platform. The SWIR hyperspectral imaging system comprised of thermoelectrically cooled SWIR camera and a spectrograph (SPECIM N25E) with a sensor array of 300×384×288 pixels and a cryogenically

cooled mercury-cadmium-telluride (MCT) detector array. Using this system, the HSI acquisition was done in the wavelength range of 953.4 - 2567.4 nm with a spectral resolution of 5.6 nm and a 30 mm focusing lens (SPECIM OLES 30).

4.4.4. Image acquisition and processing

Both Vis-NIR and SWIR systems were turned on 30 minutes prior to the acquisition of HSI to ensure thermal and temporal stability [23; 24]. The frame rate for both cameras was set to 20 frames per second (fps). The moving stage speed was chosen at 7 mm/s for both systems to achieve the optimum aspect ratio of frame rate and exposure duration [24]. For the Vis-NIR system, the exposure duration was set at 20 ms and for the SWIR system it was set at 8 ms. To obtain a black reference, the shutter was closed, whereas for white reference acquisition a 99% Spectralon reflectance standard (Labsphere, North Sutton, NH) was placed at the top of each image. Black and white reference corrections were applied after the images were acquired.

The following equation (1) was used to determine the relative reflectance R:

$$R = \frac{H-D}{W-D} \qquad (1)$$

where H stands for the original hyperspectral image and D and W stand for the black and white reference signals, respectively.

4.4.5. Image processing and spectra acquisition

For both the Vis-NIR and SWIR ranges, spectra with low signal-to-noise ratio (SNR) at the two extremes of the wavelength range were eliminated from the hypercubes. Binary masks were then used to separate the region of interest (flaxseed samples) from the background. The pixels of the region of interest were allocated a value of 1 (white), whereas pixels belonging to the undesired background were assigned a value of 0 (black). The pixels of each flaxseed sample were then

averaged to generate a representative spectrum which was saved in a spreadsheet for further analyses.

4.4.6. Multivariate data analysis

Data dimension reduction, and model construction were all accomplished using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). PCA is a data transformation approach that reduces a large number of correlated variables to a smaller number of uncorrelated orthogonal variables called principal components (PCs). PLS-DA, on the other hand, is a supervised classification algorithm that makes use of non-orthogonal latent variables (LVs). The LVs are discovered by analyzing the covariance between response variables and a design matrix that includes dummy variables that represent different dataset classes. Prior to applying PLS-DA the spectral datasets were divided into calibration and external validation sets using Kennard-Stone algorithm [37]. Spectral datasets were preprocessed utilising mathematical pre-treatments such as mean centering, first derivative, second derivative, smoothing, standard normal variate (SNV), and their combinations prior to the use of these multivariate data analysis techniques [25]. To evaluate the performance of the PLS-DA models, sensitivity, specificity, and non-error rate were assessed. Sensitivity (SENS) and specificity (SPEC) values were calculated using equation 2 and equation 3, respectively. The non-error rate was calculated as a ratio of correctly classified samples (sensitivity) to the total number of samples as shown in equation (4).

$$Sensitivity = \frac{True\ positives\ (TP)}{True\ positives\ (TP)+False\ negatives\ (FN)} \qquad (2)$$

$$Specificity = \frac{True\ negatives\ (TN)}{True\ negatives\ (TN)+False\ positives\ (FP)} \qquad (3)$$

$$Non-error\ rate = \frac{Sensitivity\ (SENS)}{Total\ number\ of\ samples\ (N)} \qquad (4)$$

Moreover, prediction models were developed for MC, FAV, protein content, and germination using partial least squares regression (PLSR). The PLSR model performance was evaluated based on the root mean square errors in calibration, cross-validation and prediction. Wavelength selection was done using the loading plots for prediction model simplification.

4.5. Results and discussion

Spectral acquisition process resulted in two datasets of 160 samples in the Vis-NIR and 162 samples in the SWIR ranges. The raw spectra of the flaxseed can be observed in Figure 4.1a and 4.1b for the Vis-NIR range and the SWIR range, respectively.

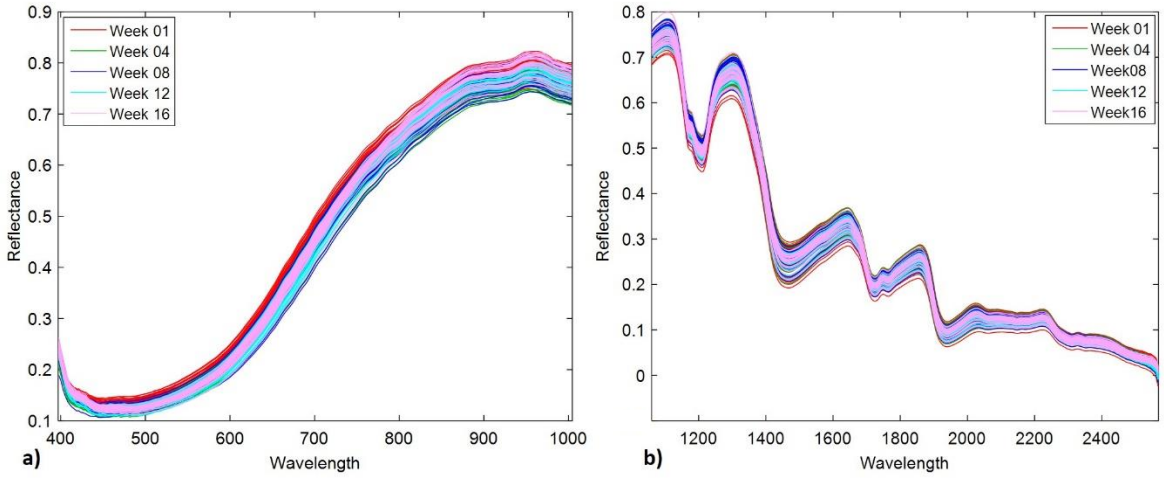

Figure 4.1. Wavelength (nm) vs Reflectance (0-1; 0 = black and 1 = white) a) Raw spectra of flaxseed in the Vis-NIR range b) Raw spectra of flaxseed in the SWIR range.

In the Vis-NIR range, the region from 650-750 nm can be associated with the red colour wavelengths and is critical for observing color changes in the samples over time [26]. On the other hand, in the SWIR region, the peaks in the second overtone region (1400 nm) and the first overtone region (1900 nm) can be associated with the moisture in the samples. The wavelength ranges from 1250-1350 nm and 1600-1800 nm are related to the presence of carbohydrates (C-H

stretching at 1329 nm) and protein content (RNH_2 structure at 1530 nm), respectively ([27]; [28]). The wavelength region from 1786-2000 nm is related to the first overtone of fatty acids (CH3- and -CH-CH-) [12].

4.5.1. PCA based data exploration

Figure 2a shows the sample/score plot of PC1 scores separated by time of storage. The PCA model was applied to a mean centered dataset of 160 flaxseed samples in the Vis-NIR range, resulting in 3 PCs explaining 98.41% of the total variance in the data. It can be observed that the PC1 scores varied over the storage period depicting clear differences on the scores of samples from each storage week (weeks 1, 4, 8, 12, and 16) as shown in figure 4.2a.

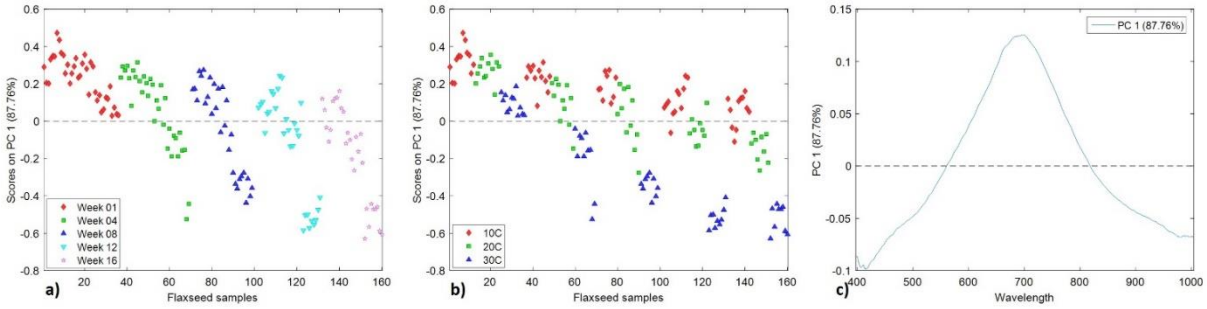

Figure 4.2. a) Sample/scores plot for PC1 separated by storage time b) Sample/scores plot for PC1 separated by storage temperature c) Loading plot for PC1.

However, the PC scores of the flaxseed samples stored at 10 °C did not change significantly over the 16-week storage period indicating minimal changes in the quality of the flaxseed at this temperature (Figure 4.2b). The highest variation in the PC1 scores was observed for the samples stored at 30 °C indicating rapid deterioration starting in the 4^{th} week of storage. Figure 2c shows the loading plot of PC1 depicting a peak around 700 nm, which can be related to the color changes of the whole flaxseed under different storage conditions. The PCA model in the Vis-NIR range did not show any groupings related to the differences in the initial moisture contents of the samples.

It could be because the major spectral peaks related to moisture content are located in the SWIR region.

Therefore, a mean centered dataset of 162 samples in the SWIR range was used to develop a second PCA model to observe groupings of the samples based on initial moisture contents (7, 8, 9, and 13%). The model yielded 7 PCs explaining 99.99% of the variance in the data. PC1 covered 95.56% of the total variance, resulting in a significant contribution towards the observation of groupings based on initial moisture contents. Figures 4.3a, 4.3b, and 4.3c depict the sample/score plot of PC1, scores plot of PC1 and PC5, and loading plot of PC1, respectively.

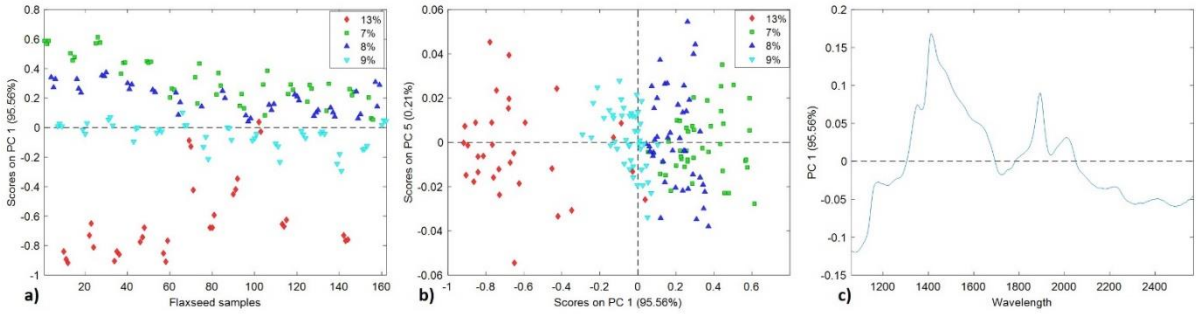

Figure 4.3. a) Sample/scores plot for PC1 separated by initial moisture content b) Scores plot for PC1 vs PC5 separated by initial moisture content c) Loading plot for PC1.

The loading plot of PC1 clearly indicates significant peaks in the 1st overtone region and the combination region associated to O-H stretch bonds. The peaks at 1400 nm and 1900 nm are associated with the moisture content of the flaxseed; hence, significantly contributing towards the classification of samples based on differences in initial moisture content. The samples possessing an initial moisture content of 13% were clearly discriminated as observed in figure 4.3b. The discrimination between flaxseed samples possessing 7% and 8% initial moisture contents was not encouraging but observable. Similar, objectives were pursued by Sundaram et al., (2012)

indicating that the regions from 1400-1440 nm and 1900-1950 nm were associated with strong NIR absorption bands for water concentration [29]. The PCA models were also evaluated for outlier detection using the Hotelling T^2 and Q residual plots. None of the samples possessed Q residual values high enough to be categorized as an outlier.

4.5.2. PLS-DA based supervised classification

From figure 4.2, it can be observed that the flaxseed samples were satisfactorily grouped in the Vis-NIR range based on storage time and temperature, therefore, PLS-DA models with different data pre-treatments were developed for the supervised classification based on these factors. Venetian blinds cross-validation with 10 data splits was used in this case. For the calibration modelling, 112 samples were used. Standard normal variate (SNV) followed by mean centering proved to be the best pre-treatment resulting in a PLS-DA model yielding highest classification accuracy. The PLS-DA calibration model resulted in 5 LVs explaining 99.84% of the total covariance in the data. Out of 5 classes (weeks 1, 4, 8, 12, 16), the samples belonging to the 4^{th} and 8^{th} weeks showed the lowest classification accuracies. The highest classification accuracies were observed for samples belonging to week 1 and week 16 of storage followed by that of week 12. The calibration statistics of the PLS-DA model for the classification of samples based on storage time are shown in Table 4.1.

Table 4.1. PLS-DA classification statistics for storage time-based classification.

Storage time-based classification									
Calibration		Actual class					N	Global	
Predicted as class		Week 1	Week 4	Week 8	Week 12	Week 16		SENS	SPEC
	Week 1	24	1	3	0	0	26	92	95
	Week 4	0	19	3	0	0	24	79	96
	Week 8	2	2	9	1	1	21	42	93
	Week 12	0	0	5	17	1	20	85	93
	Week 16	0	2	1	2	19	21	90	94
		Total					112	Non-error rate: 77.6%	

Cross validation		Actual class					N	Global	
Predicted as class		Week 1	Week 4	Week 8	Week 12	Week 16		SENS	SPEC
	Week 1	24	1	4	0	0	26	92	94
	Week 4	0	16	4	0	0	24	66	95
	Week 8	2	4	7	1	1	21	33	91

	Week 12	0	0	5	17	2	20	85	92
	Week 16	0	3	1	2	18	21	85	93
		Total					112	Non-error rate: 72.2%	

External Prediction		Actual class					N	Global	
Predicted as class		Week 1	Week 4	Week 8	Week 12	Week 16		SENS	SPEC
	Week 1	9	1	1	0	0	10	90	94
	Week 4	0	6	0	0	0	10	60	100
	Week 8	1	3	6	1	0	10	60	86
	Week 12	0	0	2	9	1	10	90	92
	Week 16	0	0	1	0	7	8	87	97
		Total					48	Non-error rate: 77.4%	

To determine the reliability of the calibration model, external prediction was done using a spectral dataset of 48 samples. Reliable prediction results were obtained with samples from week 1, week 12 and week 16 portraying high sensitivity values of 90, 90, and 87%, respectively. The non-error rates in calibration, cross-validation, and external prediction were 77.6, 72.2, and 77.4 %,

respectively. Since this classification model was developed in the wavelength range of 570-820 nm, it can reliably be deduced that this classification was attributed to the color changes of the whole flaxseeds during storage. According to the loading plots, a major peak was observed in the wavelength region of 650-750 nm, which is associated with the red region of the visible spectrum. The reason for the lower classification accuracies of week 4 and week 8 samples can be attributed to the flaxseed being stored at a high temperature (30 °C), which caused it to spoil faster and get misclassified in weeks 8 and 12, respectively. Upon observation, it became apparent that the spoiled seeds were dark brown in colour which could be correlated with higher levels of tannins in the cell pigment of flax seedcoat [30] [31].

Another supervised classification model was developed to classify the flaxseed samples based on their initial moisture content. In this regard, the SWIR wavelength range from 1000-2500 nm was used. The preprocessing of the spectral dataset included mean centering and combinations of SNV, first derivative and mean centering, which provided reliable results. The PLS-DA model with mean-centering provided the best classification accuracies for the four classes of flaxseed samples (7, 8, 9, and 13% initial MC). Table 4.2 depicts the confusion matrices in calibration, cross-validation, and prediction for the classification of flaxseed samples based on initial MC.

Table 4.2. PLS-DA classification statistics for initial moisture content-based classification.

Spectra-based classification								
Calibration		Actual class				N	Global	
Predicted as class		13%	7%	8%	9%		SENS	SPEC
	13%	25	0	0	0	25	100	100
	7%	0	22	8	0	29	76	91
	8%	0	6	16	3	27	59	90
	9%	0	1	3	29	32	91	95
	Total					113	Non-error rate: 81.5 %	
Cross validation		Actual class				N	Global	
Predicted as class		13%	7%	8%	9%		SENS	SPEC
	13%	25	0	0	0	25	100	100
	7%	0	21	10	0	29	72	88
	8%	0	7	11	7	27	41	84
	9%	0	1	6	25	32	78	91
	Total					113	Non-error rate: 72.8 %	
External Prediction		Actual class				N	Global	
Predicted as class		13%	7%	8%	9%		SENS	SPEC

	13%	7	0	0	0	7	100	100
	7%	0	10	3	0	14	71	91
	8%	0	4	11	0	14	79	88
	9%	0	0	0	13	13	100	100
	Total					48	Non-error rate: 87.5 %	

It can be observed that flaxseed samples with an initial moisture content of 13% showed 100% correct classification accuracy in calibration and cross-validation throughout the storage period. The lowest classification accuracy in calibration and cross-validation was recorded for flax samples at 8% initial moisture content with most of the samples of this class misclassified as those at 7% initial moisture content. The reliability of the calibration model was tested using external prediction where sensitivities of 100, 71, 78, and 100% were recorded for flax classes with an initial moisture content of 13, 7, 8, and 9%, respectively. The overall non-error rates in calibration, cross-validation, and prediction were 81.5, 72.8, and 87.5 %, respectively.

4.5.3. Prediction of quality parameters

The statistical values of the measured parameters of flaxseed during the 16-week storage period are reported in Table 4.3. Both PCA and PLS-DA classification models yielded reliable classification in the SWIR wavelength range with highest contributions from the second overtone and the combination band region. Since these regions are related to the O-H bonds in biological samples, SWIR wavelength range was used for developing calibration and prediction models for flaxseed moisture content.

Table 4.3. Statistical values for quality parameters of flaxseed.

Parameter	Minimum	Maximum	Mean	Standard Deviation
Moisture content	7.27	12.94	9.40	1.66
Fatty acid value	5.82	84.46	15.32	14.48
Protein content	20.45	22.19	21.47	0.28
Germination	48	100	91.56	10.43

Various data pre-treatments including mean centering, combination of SNV and mean centering, combination of SNV, 1st derivative and mean centering were used to develop calibration models. SNV was applied to minimize the effect of light scattering, which can be associated with particle size of the sample under investigation [32]. The best performance in calibration was recorded using the combination of SNV and mean centering. The model yielded an R_c^2 of 0.96 and R_{cv}^2 of 0.95 followed by root mean square error of calibration (RMSEC) of 0.33 and RMSECV of 0.37 utilizing 5 LVs. The first LV (LV1) explained 95.05% covariance of the data. The reliability of the calibration model was tested using an external prediction dataset of 48 samples. The external prediction resulted in an R_p^2 of 0.96 and RMSEP of 0.31. The loading plot of the LV1 was used for wavelength selection. The wavelength regions from 1350-1450 nm and 1860-1950 nm (Figure 4.4b) were used for developing a simplified calibration model which resulted in a total of 5 LVs with the highest covariance explained by LV1 (98.72%). After wavelength selection, the calibration model yielded R_c^2 of 0.96 and R_{cv}^2 of 0.95 with the RMSEC and RMSECV of 0.34 and 0.38, respectively (Figure 4.4a). This calibration model was validated using an external prediction, which yielded an R_p^2 of 0.97 and RMSEP of 0.27. Therefore, after wavelength selection, the model

performance improved in prediction. Figure 4 shows the scores and loading plots for the calibration of moisture content.

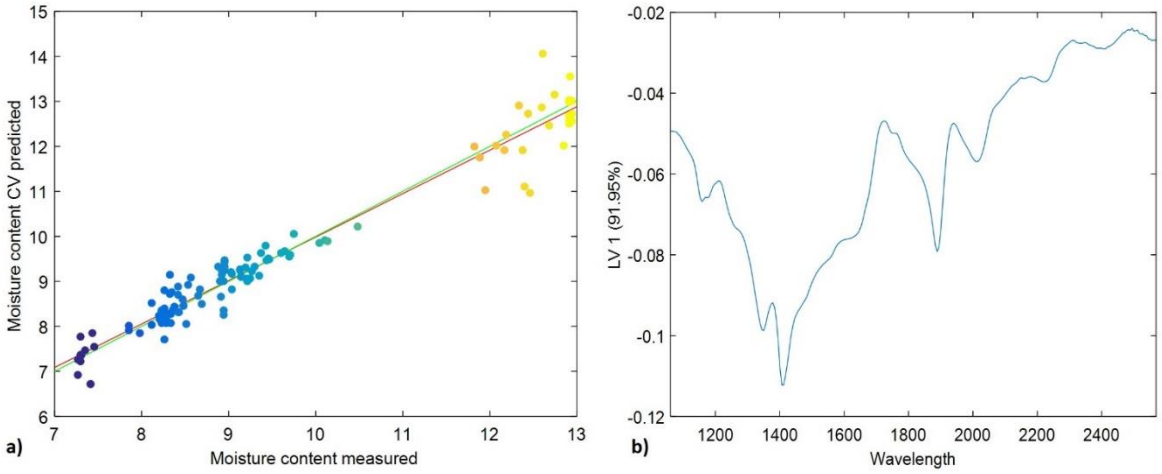

Figure 4.4. a) Calibration scores for moisture content prediction in flaxseed b) loading plot for LV1.

Calibration models were also developed for the fatty acid value which changes significantly during the storage of flaxseed. In this case, combinations of various pre-treatments were used. The model yielded most reliable results using a combination of SNV, smoothing, and mean centering. To reduce the scattering effects to better predict the fatty acid content, SNV was used as a data pre-treatment [33]. The calibration model was developed using 9 LVs with the first LV explaining 91.77% of the data's co-variance. The model resulted in an R_c^2 of 0.91 and R_{cv}^2 of 0.88 followed by RMSEC and RMSECV of 4.93 and 5.68, respectively. For the validation of the model, an external prediction set of 48 samples was used yielding an R_p^2 of 0.76 and RMSEP of 6.44. The loading plots of LV1 (Figure 4.5d) showed that C-H bond which is an essential component of the fatty acid molecule shows absorption peaks at 1200, 1400, 1750, 2310, and 2340 nm. The C-H first overtone of the fatty acids (CH3- and -CH-CH-) is associated with the wavelength range of

1786-2000 nm [34]. Moreover, the absorption peaks at 1923 and 1961 nm can be linked to the O-H first overtone [35]. The wavelength range of 1450-1470 nm has been linked with H-bonds from the OH groups. Oliveira et al. (2020) associated the band absorption at 1715 nm with fat and fatty acids [36]. Figure 5 shows the calibration scores, and loadings plots for the prediction of fatty acid content in flaxseed. It can be observed that the FAV content significantly increased for samples with 13% initial moisture content (Figure 4.5a) stored at 20 and 30 °C (Figure 4.5b). The calibration scores are separated by time of storage as shown in Figure 4.5c. The PLSR models did not depict reliable prediction results for protein content and germination.

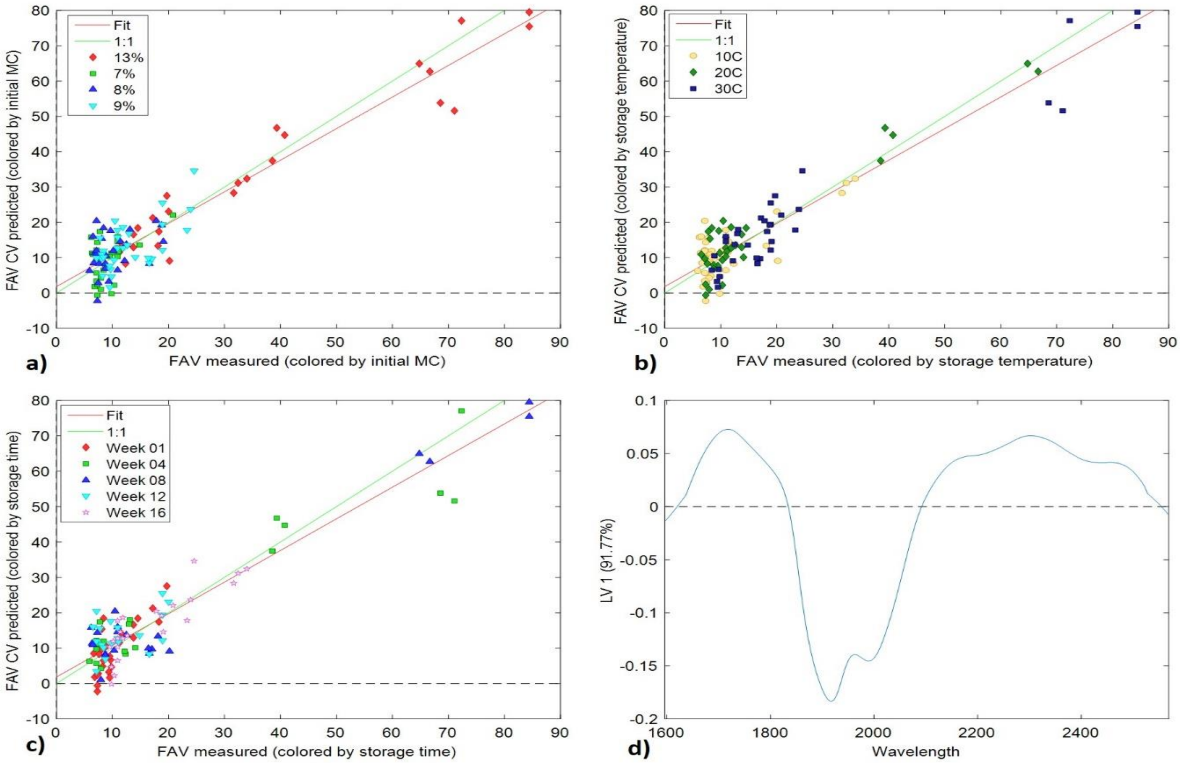

Figure 4.5. a) Calibration scores for FAV prediction in flaxseed colored by initial MC b) Calibration scores for FAV prediction in flaxseed separated by storage temperature c) Calibration scores for FAV prediction in flaxseed separated by storage time d) loading plot for LV1.

4.6. Conclusions

Discrimination of flaxseed based on storage time and temperature was observed in the Vis-NIR region. This information can be used by the industry to predict MC fluctuations during storage for efficient oil extraction. The classification of flaxseed based on storage conditions help to predict oil quality to prevent degradation, to reduce losses for the industries and will help farmers to get better value for their crop. In this case, the wavelength region from 650-750 nm associated with color played a significant role in discriminating flax. The highest misclassification was observed for flax samples from the 4^{th} and 8^{th} weeks of storage. SWIR region was useful in classification of flaxseed based on initial moisture content with major peaks in the second overtone region (1400 nm) and combination region (1900 nm). Samples at 13% initial moisture content depicted 100% correct classification. Though misclassification was observed for samples at 7, 8, and 9% initial moisture contents. PLSR models yielded reliable models for the prediction of moisture content and fatty acid composition for stored flaxseed. The prediction models for protein content and germination were not satisfactory. Conclusively, NIRS can be a useful tool to assist producers, traders, and food processors to take timely logistical decisions for optimum utilization of flax for end uses. The future endeavours will include the prediction of flax quality parameters utilizing flax flour instead of intact seeds.

4.7. Acknowledgements

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COMPETING INTERESTS

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in the manuscript.

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CHAPTER 5. Thesis summary and overall conclusion

This research led to the development of a safe storage guideline chart for flaxseed grown in the Canadian Prairies. Seed germination rate and FAV were affected by storage variable such as moisture content, storage temperature, and storage period. The storage variables and flaxseed germination rate were inversely proportional, but the FAV increased with increased of storage variables. Throughout the storage period, germination rate was observed above 80 % for samples with 7, 8, and 9 % initial moisture content when stored at 10 and 20 °C. The seeds may be stored for at least 16 weeks at 7, 8, and 9 % moisture content at 10 and 20 °C with minimal quality loss. When stored at 20 and 30 °C, germination rate of seeds with 13% MC was significantly reduced. Flaxseed held at 13 % moisture lost germination in the 12^{th} and 11^{th} weeks when stored at 20 and 30 °C, respectively. Flaxseed with MC, 13% or higher must be dried to at least 8 % when stored at 20 and 30 °C within three weeks of storage, which is not recommended but necessary in exceptional circumstances. Visible mould was observed in the first 4 weeks of storage at temperatures 20 °C and above with a MC of 13 %. To preserve high seed quality, flaxseed should be maintained below 9% moisture content and at temperatures below 20°C for extended storage periods. The Vis-NIR region revealed flaxseed discrimination dependent on storage duration and temperature. For effective oil extraction, industries can use this knowledge to estimate the initial MC changes during storage. The classification of flaxseed based on storage conditions aids in the prediction of oil quality and the prevention of deterioration, making it more lucrative for industries and farmers. The color-related wavelength range of 650-750 nm played a key role in distinguishing flax. Seed samples from the 4^{th} and 8^{th} weeks of storage had the most misclassification. With large peaks in the second overtone region (1400 nm) and combination region, the SWIR region proved beneficial in classifying flaxseed depending on initial moisture content (1900 nm). At a MC of 13%, samples were classified correctly 100%. Misclassification was reported for samples with initial moisture values of 7, 8, and 9%. For the prediction of moisture content and fatty acid composition in stored flaxseed, PLSR models were found to be reliable. Protein content and germination prediction models were both unsatisfactory. To summarise, NIRS can be a valuable tool for assisting growers, farmers, and food processors in making timely logistical decisions for the most efficient use of flax for end applications.

CHAPTER 6. Recommendations for future research

- To establish the influence of storage conditions quality factors like nutritional changes, milling, and baking quality of flaxseed flours should be examined.
- To learn more about the impact of long-term storage on seed quality, the study should be extended.
- High Performance Liquid Chromatography (HPLC) can be used to investigate changes in the fatty acid content of flaxseed over time.
- FTIR analysis of flaxseed can be used to identify fat and protein oxidation and develop prediction models.
- Future research should focus on predicting seed quality metrics using flaxseed flour rather than the whole seed.

APPENDIX

Table 7.1. Moisture content of samples stored ta 10 ℃

Storage week	Replicate	7%	8%	9%	13%
0	1	7.41	8.28	8.92	12.91
	2	7.3	8.26	8.95	12.91
	3	7.27	8.23	9.13	12.94
	Mean	7.33	8.27	8.99	12.92
	Sd	0.07	0.03	0.11	0.02
4	1	7.85	8.12	9.43	12.07
	2	7.45	8.42	9.03	11.91
	3	7.31	8.27	9.14	11.99
	Mean	7.53	8.27	9.20	11.99
	Sd	0.28	0.15	0.21	0.08
8	1	8.30	8.42	9.77	12.17
	2	8.35	8.32	9.52	12.10
	3	7.85	8.44	9.94	11.89
	Mean	8.16	8.40	9.74	12.05
	Sd	0.28	0.06	0.21	0.15
12	1	8.52	8.90	9.75	12.19
	2	8.35	8.94	9.64	12.23
	3	8.03	8.88	10.10	11.94
	Mean	8.30	8.91	9.83	12.12
	Sd	0.25	0.03	0.24	0.16
16	1	8.56	9.19	10.13	12.44
	2	8.23	9.14	10.05	12.68
	3	8.03	9.04	10.48	12.59
	Mean	8.27	9.12	10.22	12.57
	Sd	0.27	0.08	0.23	0.12

Table 7.2. Moisture content of samples stored at 20 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	7.41	8.28	8.92	12.91
	2	7.3	8.26	8.95	12.91
	3	7.27	8.23	9.13	12.94
	Mean	7.33	8.26	8.99	12.92
	Sd	0.07	0.03	0.11	0.02
4	1	7.75	8.03	9.06	12.74
	2	7.35	8.10	8.95	12.92
	3	7.44	8.11	9.09	12.34
	Mean	7.51	8.08	9.03	12.66
	Sd	0.21	0.21	0.07	0.30
8	1	8.34	8.34	9.15	12.84
	2	7.98	8.52	9.37	12.44
	3	8.11	8.77	9.25	12.38
	Mean	8.14	8.54	9.26	12.55
	Sd	0.18	0.21	0.11	0.25
12	1	8.38	8.60	9.28	11.81
	2	8.43	8.42	9.47	11.39
	3	8.35	8.65	9.22	11.56
	Mean	8.39	8.56	9.32	11.59
	Sd	0.04	0.12	0.13	0.21
16	1	8.47	8.64	9.70	11.76
	2	8.20	8.67	9.70	11.29
	3	8.37	9.62	9.60	11.52
	Mean	8.35	8.97	9.66	11.52
	Sd	0.14	0.56	0.06	0.24

Table 7.3. Moisture content of samples stored at 30 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	7.41	8.28	8.92	12.91
	2	7.3	8.26	8.95	12.91
	3	7.27	8.23	9.13	12.94
	Mean	7.33	8.27	8.99	12.92
	Sd	0.07	0.03	0.11	0.02
4	1	7.97	8.18	8.54	12.39
	2	7.95	8.48	8.66	12.46
	3	7.90	8.33	8.65	12.61
	Mean	7.94	8.33	8.62	12.49
	Sd	0.04	0.15	0.07	0.11
8	1	8.67	8.91	9.21	11.82
	2	8.21	8.88	9.27	11.95
	3	8.54	8.83	9.14	11.75
	Mean	8.47	8.87	9.21	11.84
	Sd	0.24	0.04	0.06	0.10
12	1	8.54	8.74	9.35	11.66
	2	8.14	8.94	9.30	11.37
	3	8.34	8.93	9.20	11.48
	Mean	8.34	8.87	9.28	11.50
	Sd	0.20	0.12	0.07	0.15
16	1	8.85	8.70	9.45	11.29
	2	8.32	9.04	9.42	11.16
	3	8.25	8.95	9.47	11.11
	Mean	8.47	8.89	9.45	11.19
	Sd	0.33	0.18	0.02	0.10

Table 7.4. FAV of samples stored at 10 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	7.09	6.70	8.26	10.72
	2	6.82	7.36	8.13	11.23
	3	6.32	7.49	8.13	11.49
	Mean	6.73	7.17	8.17	11.14
	Sd	0.32	0.34	0.06	0.32
4	1	7.10	6.85	8.39	12.27
	2	7.88	5.81	7.10	12.26
	3	7.10	7.10	8.38	13.56
	Mean	7.35	6.56	7.93	12.68
	Sd	0.36	0.56	0.61	0.61
8	1	6.32	8.78	8.65	20.16
	2	6.20	7.88	8.91	19.51
	3	7.36	7.10	9.05	18.06
	Mean	6.60	7.89	8.87	19.22
	Sd	0.52	0.68	0.16	0.87
12	1	7.09	7.10	7.75	20.01
	2	7.75	7.10	8.14	20.65
	3	6.19	6.46	8.39	19.50
	Mean	6.98	6.88	8.09	20.05
	Sd	0.63	0.30	0.26	0.47
16	1	9.29	10.19	9.81	32.44
	2	9.43	10.85	10.97	33.97
	3	9.81	11.36	10.98	31.66
	Mean	9.51	10.79	10.57	32.67
	Sd	0.21	0.48	0.55	0.96

Table 7.5. FAV of sample stored at 20 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	6.84	7.62	9.55	13.70
	2	7.23	8.39	9.56	14.46
	3	7.36	8.14	9.81	13.70
	Mean	7.14	8.04	9.64	13.94
	Sd	0.21	0.32	0.12	0.36
4	1	7.10	6.71	13.04	40.81
	2	7.10	6.84	14.06	39.40
	3	7.75	7.10	13.30	38.52
	Mean	7.31	6.88	13.46	39.56
	Sd	0.30	0.16	0.43	0.94
8	1	6.58	7.75	10.33	64.76
	2	8.78	9.17	10.46	62.64
	3	7.87	10.19	10.20	66.65
	Mean	7.69	8.98	10.33	64.67
	Sd	0.90	1.00	0.10	1.64
12	1	9.81	9.82	11.10	-
	2	10.98	8.78	10.97	
	3	11.10	9.69	10.85	
	Mean	10.61	9.42	10.97	-
	Sd	0.58	0.45	0.10	-
16	1	10.85	10.08	11.87	-
	2	10.34	10.33	11.88	
	3	10.59	10.59	12.65	
	Mean	10.59	10.33	12.13	-
	Sd	0.21	0.21	0.36	-

Table 7.6. FAV of samples stored at 30 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	8.27	8.78	9.68	17.19
	2	8.40	9.29	9.81	19.76
	3	8.14	8.91	9.56	18.34
	Mean	8.27	8.99	9.68	18.40
	Sd	0.10	0.21	0.10	1.05
4	1	10.98	12.66	12.91	68.51
	2	11.10	13.17	12.92	71.07
	3	10.98	12.14	12.92	72.36
	Mean	11.02	12.65	12.92	70.63
	Sd	0.05	0.41	0.01	1.59
8	1	10.97	12.40	16.53	84.46
	2	10.98	12.53	17.06	84.36
	3	10.72	12.91	16.41	83.33
	Mean	10.89	12.61	16.67	84.05
	Sd	0.12	0.21	0.28	0.501
12	1	14.47	16.66	18.98	-
	2	15.75	16.53	18.86	
	3	14.86	16.27	18.86	
	Mean	15.02	16.49	18.90	-
	Sd	0.53	0.16	0.05	-
16	1	20.15	17.83	23.25	-
	2	20.80	18.73	24.53	
	3	21.17	19.10	23.89	
	Mean	20.70	18.54	23.88	-
	Sd	0.42	0.53	0.52	-

Table 7.7. Germination of samples stored at 10 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	100	96	100	96
	2	96	100	96	96
	3	100	100	100	100
	Mean	98.64	98.64	98.64	97.31
	Sd	2.30	2.30	2.30	2.30
4	1	96	96	96	96
	2	96	100	100	96
	3	100	100	100	100
	Mean	97.31	98.64	98.64	97.31
	Sd	2.30	2.30	2.30	2.30
8	1	100	96	96	92
	2	96	100	100	92
	3	100	96	96	92
	Mean	98.64	97.31	97.31	92
	Sd	2.30	2.30	2.30	0
12	1	96	96	92	88
	2	96	96	96	88
	3	100	92	96	84
	Mean	97.31	94.64	94.64	86.64
	Sd	2.30	2.30	2.30	2.30
16	1	92	92	84	72
	2	92	92	88	76
	3	88	92	84	72
	Mean	90.64	92	85.31	73.30
	Sd	2.30	0	2.30	2.30

Table 7.8. Germination of samples stored at 20 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	100	96	100	96
	2	96	100	96	96
	3	100	100	100	100
	Mean	98.64	98.64	98.64	97.31
	Sd	2.30	2.30	2.30	2.30
4	1	100	92	96	84
	2	96	96	100	88
	3	92	96	96	84
	Mean	95.94	94.64	97.31	85.31
	Sd	4	2.30	2.30	2.30
8	1	96	96	96	60
	2	96	96	92	56
	3	92	96	96	60
	Mean	94.64	96	94.64	58.63
	Sd	2.30	0	2.30	2.30
12	1	96	96	96	8
	2	96	92	92	8
	3	96	96	96	4
	Mean	96	94.64	94.64	6.34
	Sd	0	2.30	2.30	2.30
16	1	88	88	88	0
	2	92	88	84	0
	3	88	92	88	0
	Mean	89.31	89.31	86.64	0
	Sd	2.30	2.30	2.30	0

Table 7.9. Germination of samples stored at 30 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	100	96	100	96
	2	96	100	96	96
	3	100	100	100	100
	Mean	98.64	98.64	98.64	97.31
	Sd	2.30	2.30	2.30	2.30
4	1	96	96	96	84
	2	96	92	100	84
	3	100	96	96	80
	Mean	97.31	94.64	97.31	82.64
	Sd	2.30	2.30	2.30	2.30
8	1	100	92	96	72
	2	96	96	96	72
	3	100	96	96	76
	Mean	98.64	94.64	96	73.30
	Sd	2.30	2.30	0	2.30
12	1	96	92	84	0
	2	92	84	88	0
	3	92	92	84	0
	Mean	93.31	89.25	85.31	0
	Sd	2.30	4.61	2.30	0
16	1	84	76	52	0
	2	80	76	56	0
	3	80	72	56	0
	Mean	81.31	74.64	54.63	0
	Sd	2.30	2.30	2.30	0

Table 7.10. Protein content of samples stored at 10 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	21.66	21.79	21.50	21.35
	2	21.29	21.33	21.42	21.34
	3	20.94	21.91	21.36	21.20
	Mean	21.30	21.67	21.43	21.30
	Sd	0.29	0.25	0.05	0.06
4	1	21.61	21.56	21.62	21.53
	2	21.57	21.56	21.51	21.35
	3	21.48	21.47	21.28	21.37
	Mean	21.55	21.53	21.47	21.42
	Sd	0.05	0.04	0.14	0.08
8	1	21.65	21.71	21.54	21.58
	2	22.16	21.66	21.45	21.52
	3	21.72	21.43	21.39	21.58
	Mean	21.84	21.60	21.46	21.56
	Sd	0.22	0.12	0.06	0.02
12	1	21.38	21.20	21.14	21.28
	2	21.17	21.41	20.82	21.16
	3	21.34	21.18	21.39	21.14
	Mean	21.30	21.26	21.12	21.20
	Sd	0.09	0.10	0.23	0.06
16	1	21.50	21.18	21.30	21.15
	2	21.25	21.05	21.29	21.11
	3	20.90	21.26	20.44	21.35
	Mean	21.22	21.16	21.01	21.20
	Sd	0.24	0.08	0.40	0.10

Table 7.11. Protein content of samples stored at 20 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	21.59	21.39	21.30	21.32
	2	21.22	21.33	21.20	21.32
	3	21.40	21.30	21.36	21.15
	Mean	21.40	21.34	21.29	21.26
	Sd	0.15	0.03	0.06	0.07
4	1	21.23	21.39	21.50	21.60
	2	21.47	21.66	21.31	21.48
	3	21.47	21.19	21.34	21.32
	Mean	21.39	21.41	21.38	21.47
	Sd	0.11	0.19	0.08	0.11
8	1	21.83	21.86	21.69	21.45
	2	21.94	21.65	22.18	21.75
	3	21.50	21.73	22.18	21.47
	Mean	21.76	21.74	22.02	21.56
	Sd	0.18	0.08	0.23	0.13
12	1	21.60	21.81	21.73	21.29
	2	21.82	21.75	21.97	21.44
	3	21.82	21.64	21.83	21.42
	Mean	21.75	21.73	21.84	21.38
	Sd	0.10	0.07	0.09	0.06
16	1	21.29	20.81	21.22	-
	2	21.44	21.19	21.20	
	3	21.42	21.38	21.38	
	Mean	21.38	21.12	21.27	-
	Sd	0.06	0.23	0.07	-

Table 7.12. Protein content of samples stored at 30 °C

Storage week	Replicate	7%	8%	9%	13%
0	1	21.48	21.45	21.51	21.50
	2	21.65	21.51	21.63	21.31
	3	21.66	21.29	21.48	21.54
	Mean	21.60	21.42	21.54	21.45
	Sd	0.08	0.09	0.06	0.09
4	1	21.81	21.88	20.87	21.90
	2	21.61	21.49	21.66	21.44
	3	21.55	21.84	21.61	21.85
	Mean	21.66	21.74	21.68	21.73
	Sd	0.11	0.17	0.36	0.20
8	1	21.47	21.69	21.48	21.52
	2	21.72	21.64	21.63	21.70
	3	21.46	21.24	21.64	21.60
	Mean	21.55	21.52	21.58	21.61
	Sd	0.12	0.20	0.07	0.07
12	1	21.66	21.80	21.78	-
	2	21.56	21.68	21.71	
	3	21.60	21.60	21.63	
	Mean	21.61	21.69	21.71	-
	Sd	0.04	0.08	0.06	-
16	1	21.40	22.09	21.45	-
	2	21.16	20.97	21.26	
	3	21.35	20.47	21.01	
	Mean	21.30	21.16	21.24	-
	Sd	0.10	0.67	0.18	-

Table 7.13. Tests between subject's effects

Independent variable	Dependent variable	Df	F	Sig.
Temperature	MC	2	1.512	.225
	Germination	2	229.345	.000
	FAV	2	6355.332	.000
ERH	MC	3	4162.329	.000
	Germination	3	303.749	.000
	FAV	3	13118.184	.000
Storage Period	MC	4	76.823	.000
	Germination	4	340.757	.000
	FAV	4	1984.715	.000
Temperature * ERH	MC	6	16.264	.000
	Germination	6	39.807	.000
	FAV	6	1880.448	.000
Temperature * Storage Period	MC	8	1.737	.097
	Germination	8	46.432	.000
	FAV	8	368.586	.000
ERH * Storage Period	MC	12	24.432	.000
	Germination	12	56.606	.000
	FAV	12	1035.737	.000
Temperature * ERH * Storage Period	MC	20	3.369	.000
	Germination	20	13.111	.000
	FAV	20	239.327	.000

Table 7.14. Multiple comparisons based on storage period

Parameter	**Post hoc test**	**Storage period (in weeks)**	**Storage period (in weeks)**	**Standard deviation**	**Sig.**
Moisture content	Tukey HSD	0.00	4.00	.04003	.401
			8.00	.04003	.000
			12.00	.04199	.000
			16.00	.04199	.981
		4.00	.00	.04003	.401
			8.00	.04003	.000
			12.00	.04199	.060
			16.00	.04199	.174
		8.00	.00	.04003	.000
			4.00	.04003	.000
			12.00	.04199	.000
			16.00	.04199	.000
		12.00	.00	.04199	.000
			4.00	.04199	.060
			8.00	.04199	.000
			16.00	.04385	.000
		16.00	.00	.04199	.981
			4.00	.04199	.174
			8.00	.04199	.000
			12.00	.04385	.000
	LSD	.00	4.00	.04003	.081
			8.00	.04003	.000
			12.00	.04199	.000
			16.00	.04199	.580
		4.00	.00	.04003	.081
			8.00	.04003	.000

			12.00	.04199	.008
			16.00	.04199	.027
		8.00	.00	.04003	.000
			4.00	.04003	.000
			12.00	.04199	.000
			16.00	.04199	.000
		12.00	.00	.04199	.000
			4.00	.04199	.008
			8.00	.04199	.000
			16.00	.04385	.000
		16.00	.00	.04199	.580
			4.00	.04199	.027
			8.00	.04199	.000
			12.00	.04385	.000
Germination	Tukey HSD	.00	4.00	.54917	.000
			8.00	.54917	.000
			12.00	.57597	.000
			16.00	.57597	.000
		4.00	.00	.54917	.000
			8.00	.54917	.000
			12.00	.57597	.003
			16.00	.57597	.000
		8.00	.00	.54917	.000
			4.00	.54917	.000
			12.00	.57597	.000
			16.00	.57597	.000
		12.00	.00	.57597	.000
			4.00	.57597	.003
			8.00	.57597	.000
			16.00	.60159	.000

		16.00	.00	.57597	.000
			4.00	.57597	.000
			8.00	.57597	.000
			12.00	.60159	.000
	LSD	.00	4.00	.54917	.000
			8.00	.54917	.000
			12.00	.57597	.000
			16.00	.57597	.000
		4.00	.00	.54917	.000
			8.00	.54917	.000
			12.00	.57597	.000
			16.00	.57597	.000
		8.00	.00	.54917	.000
			4.00	.54917	.000
			12.00	.57597	.000
			16.00	.57597	.000
		12.00	.00	.57597	.000
			4.00	.57597	.000
			8.00	.57597	.000
			16.00	.60159	.000
		16.00	.00	.57597	.000
			4.00	.57597	.000
			8.00	.57597	.000
			12.00	.60159	.000
FAV	Tukey HSD	.00	4.00	.16173	.000
			8.00	.16173	.000
			12.00	.16963	.000
			16.00	.16963	.000
		4.00	.00	.16173	.000
			8.00	.16173	.000

			12.00	.16963	.000
			16.00	.16963	.000
		8.00	.00	.16173	.000
			4.00	.16173	.000
			12.00	.16963	.000
			16.00	.16963	.000
		12.00	.00	.16963	.000
			4.00	.16963	.000
			8.00	.16963	.000
			16.00	.17717	.000
		16.00	.00	.16963	.000
			4.00	.16963	.000
			8.00	.16963	.000
			12.00	.17717	.000
	LSD	.00	4.00	.16173	.000
			8.00	.16173	.000
			12.00	.16963	.000
			16.00	.16963	.000
		4.00	.00	.16173	.000
			8.00	.16173	.000
			12.00	.16963	.000
			16.00	.16963	.000
		8.00	.00	.16173	.000
			4.00	.16173	.000
			12.00	.16963	.000
			16.00	.16963	.000
		12.00	.00	.16963	.000
			4.00	.16963	.000
			8.00	.16963	.000
			16.00	.17717	.000

		16.00	.00	.16963	.000
			4.00	.16963	.000
			8.00	.16963	.000
			12.00	.17717	.000