DEVELOPMENT OF SAFE STORAGE GUIDELINES FOR KABULI CHICKPEAS (*CICER ARIETINUM. L)*

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ABSTRACT

Safe storage guidelines are key to avoid the spoilage of any crop and the subsequent economic losses that occur during storage. The guidelines assist farmers to schedule appropriate post-harvest measures and ensures quality during storage is maintained during storage of agricultural commodities. The present research work is aimed at developing safe storage guidelines for Kabuli chickpeas. The storage variables taken into consideration were storage temperature, relative humidity, and storage time. The chickpeas were stored at three temperatures (10, 20, and 30℃) and moisture contents of 9, 11, 13, and 15% in the relative humidity (RH) range of ~55 to 95% for 16 weeks. The relative humidity was maintained by four salt solutions (Mg (NO3)2), NaNO2, NaCl, KNO3,). The quality index for the stored crop was germination, change in moisture content, fatty acid value (FAV), visible mold, and protein analysis.

Statistically, all the storage variables (RH, storage period, and storage temperature) showed a significant effect (α=0.05) on seed germination, initial moisture content, and FAV. The mould appeared after the 5^{th} week of storage in the samples stored at 30 ℃ with high initial moisture content (15%). There was no significant change in the protein content of the chickpeas over the period of storage. Based on germination loss, it was concluded that chickpeas are safe to store at 10 and 20 ℃ at lower moisture contents of 9, 11, and 13%. Seed quality and viability during long-term storage can be maintained by storing chickpeas at temperatures below 20 ℃.

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1 INTRODUCTION

Fulfilling the dietary requirements of a growing population remains a major concern worldwide. Nutritional preferences of today's consumers have led to the evolution of agricultural systems emphasizing the need to make them sustainable for future demands. Currently, more than one-third of the agricultural production is lost or wasted due to inefficient postharvest operations with maximum losses occurring during the storage of these commodities (Kumar and Kalita 2017) . Specifically, in Canada, oilseeds, cereals, and legume crops are being grown to meet the rising consumer demands and for export.

Since ancient times, leguminous crops have been serving as a nutritious source of food and helping the farming systems through nitrogen fixation. They are composed of protein, fiber, carbohydrate, iron, magnesium, copper, zinc, vitamins, manganese and phosphorous. Legumes being plants, they are cholesterol-free, naturally have less fat value, and are practically free of saturated fat (Polak et al. 2015). Production of pulses assists in fixing the atmospheric nitrogen enriching soil fertility and thereby increasing the productivity of the subsequent crop or cropping system (Reddy 2011). Canadian agriculture has witnessed distinct evolution as the area under pulse cultivation has significantly increased in the last decade at 3.5 million hectares per year (Saskatchewan Pulse growers 2020). Over 75% of the annual Canadian pulse production is exported worldwide (CIGI. 2000).

Chickpea (*Cicer Arietinum.L*) belongs to Fabaceae family and is commonly known as garbanzo beans, having a high nutritional profile and protein content. Chickpeas contain 19-21% protein, 17% dietary fibre, 6% lipid content and 60% carbohydrates (Wallace et al. 2016, Manickavasagan and Thirunathan 2020). Apart from playing a beneficial role glucose and insulin regulation along with weight management, chickpea-based foods also have a positive impact on some markers of cardiovascular diseases (Wallace et al. 2016). Commercial production of chickpeas in Canada began in 1995 (CIGI 2000). The exports of Canadian chickpea increased by 43% from 2019-2020 to 150 Kt in 2020-2021 (Steve Lavergne and Fred Oleson 2021). The Food and agriculture organisation (FAO) statistics reported that Canada was the third leading chickpea exporter in 2016 with export of 137,055 million tons (Rawal and Navarro 2018).

With increasing production and export, it is imperative that the quality of the crop is maintained. If left unmonitored, the produce is bound to lose its nutritional quality and eventually decay. To

properly preserve nutritional quality of stored harvest and prolong its shelf life, safe storage guidelines are of utmost importance. If preventive and corrective measures are not taken at the right time, abiotic and biotic factors may lead to deterioration of grains. Abiotic factors that cause deterioration include moisture content and temperature whereas biotic factors comprise of arthropods, fungi, and occasionally, rodents and birds (Fleurat-Lessard 2016).

During storage, moisture content, temperature, and equilibrium relative humidity (ERH) are the major contributing factors that influence deterioration (Chidananda et al. 2014). The moisture content of chickpeas at harvesting stage is high (≤18%), therefore it is important to dry them prior to storage. The crop isn't dried properly before going into storage, its high moisture content can lead to the development of hotspots in storage bins favoring the growth of microorganisms (Zomorodian et al. 2011). Relative humidity (RH) of the environment also affects the quality parameters of the stored crop. Undesirable changes occur at high RH (≥ 70%), altering the properties of crops and leading to mold growth. On the other hand, high temperatures can lead to increased respiration in pulses causing favourable conditions for fungal infestations. Optimal post-harvest practices and storage conditions are a prerequisite to ensure longevity of seeds in storage. Hence, controlling the moisture content, relative humidity, and temperature can assist in preserving the quality and nutritional profiles of stored commodities (Saskatchewan Pulse growers 2020).

Efficient storage management strategies aid farmers and managers of commercial storage facilities plan ahead and make informed decisions to maximize the shelf life and nutritional quality of their stored commodities. To this end, storage guidelines for various cereals, oilseed and pulses have already been developed by researchers e.g., soybeans (Diaz-Contreras et al. 2021), canola (Sathya et al. 2009), rye (Sathya et al. 2008) rye (Rajarammanna et al. 2010), wheat (Nithya et al. 2011), pinto beans (Rani et al. 2013), hemp (Jian et al. 2019), black grams (Esther et al. 2015) including Australian desi chickpeas (Cassells and Caddick 2002). However, there is no data available for safe storage guidelines for Kabuli chickpeas. This knowledge gap needs to be addressed for assisting this crop of high economic importance. Therefore, the objective of this study was formulated to develop safe storage guidelines for Canadian grown Kabuli chickpeas.

2 REVIEW OF LITERATURE

2.1 Legumes

Legumes are the family (Fabaceae) of plants, fruits, and seeds that serve as most common staple diets globally. Having high nutritional profiles, legumes crops fulfil the requirement of vegetable as well as protein foods. Legume crops can significantly contribute towards poverty and hunger reduction, human health and nutritional improvement, and enhancement of ecosystem resilience for targeted populations (Akibode and Maredia 2011). When protein in legumes are combined with grain foods in a meal, essential amino acids intake also takes place (Wallace et al. 2016). Being capable of fetching higher prices as compared to cereals and serving as a feed crop, legumes are increasingly grown to supplement growers' income (Akibode and Maredia 2011).

Pulses are edible seeds harvested from certain leguminous plants. They are environmentally sustainable as they required less water/irrigation, are relatively inexpensive to produce, and are in demand globally. Total carbohydrate, niacin, thiamine, fat, riboflavin and vitamin B6 content are the similar in pulses and cereals. However, the protein content in pulses is two times higher as compared to cereals along with higher folate, iron, magnesium, potassium, and zinc (Singh 2017). Their fat content is significantly lower as compared to crops such as soybeans and peanuts. Pulses can be consumed in a variety of ways, such as ground, whole, split, or fractionated into fibers, starches, and proteins (Rawal and Navarro 2018).

2.2 Worldwide pulse production

Considering regional differences in terms of agricultural productivity, consumption habits, end markets, processing capabilities, and supply chain systems globally, the international pulse market can be best described as 'diverse and complex' (Rawal and Navarro 2018). There has been a substantial change in the global economy of pulses in the last fifteen years. There was a 63% increase in pulse production from 1998 to 2018, globally. The production of pulses was estimated to be 92.4 million tons (MT) in 2018 worldwide. Significant rise in pulse production was achieved due to the production of chickpeas (>8.3 million MT), common beans (>14 million MT), cowpeas (>3.5 million MT), lentils and pigeon peas (>3.6 million MT each), and dry peas (>1.2 million MT) (Xia, 2020). South Asia and sub-Saharan Africa produces about half of the world pulse production (Xia 2020). Various countries globally produce the beans while lentils production is highest in India, Turkey, and Canada. Peas are mainly harvested in Australia, Canada, and the United States while chickpea have a dominance in India. For thousands of years, pulses have been

a central component of people's diet in South Asia, North Africa, Sub-Saharan Africa, and the Middle East. These regions also happen to be the world's largest consumers of pulses. Owing to the size and density of its population, South Asia drives significant segments of the pulse markets. North American farmers primarily grow three types of pulses namely, dry peas, dry beans, and lentils, while a small amount of acreage is allocated to growing chickpeas and cowpeas (black-eyed peas) (Rawal and Navarro 2018).

2.3 Pulse trends in Canada

Despite the dominance of wheat and canola cultivation in Canada, the pulse seeded area has significantly increased since the 1980s. Several factors that have contributed towards the increase in pulse production. Firstly, the favourable Canadian prairie soil and climate conditions and growth of processing facilities. Secondly, research on developing new varieties having short growing season resisting lodging or disease and agronomic and economic benefits when planted in rotation with other field crops. According to the census of agriculture, pulse production in Canada increased from 7236.7 thousand tonnes in 2019-2020 to 8007.1 thousand tonnes in 2020-2021 (Steve Lavergne and Fred Oleson 2021). Canada exports pulses to over a hundred countries, but the main export destinations are Turkey (for lentils and chickpeas), the United States (for dry beans), and India and China (for dry peas). Despite their nutritional benefits and worldwide

popularity, the domestic consumption of pulses in Canada has remained low with only 13% of Canadian population reported to consume pulses or their derivatives (Bekkering 2014).

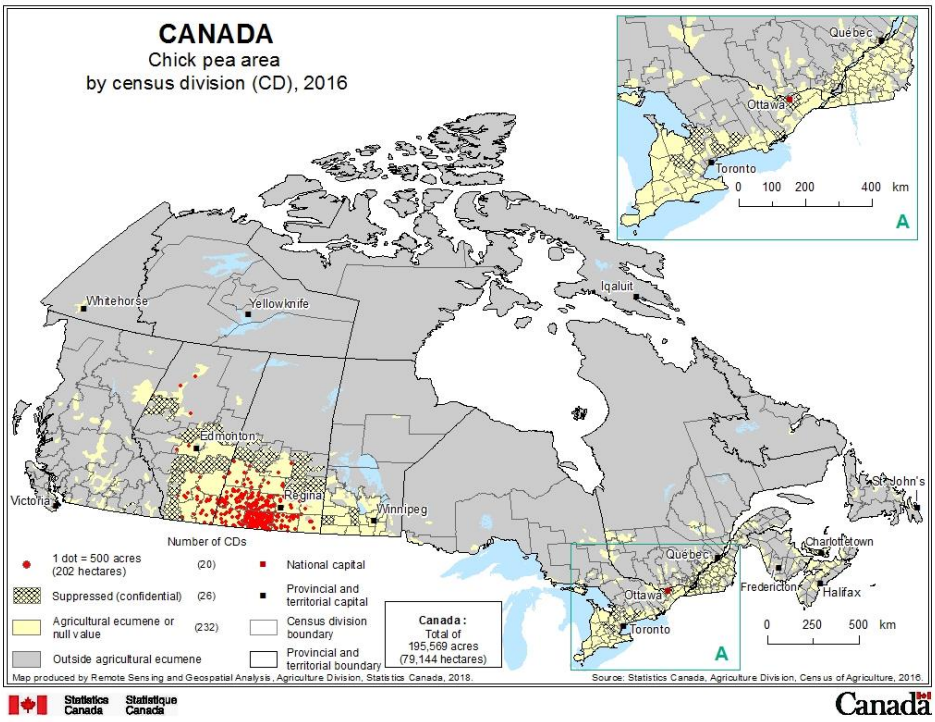

Figure 1 Map of Canada showing chickpea production area (Statistics Canada 2016)

2.4 Chickpeas

Chickpea (*Cicer arietinum.L*), commonly referred as garbanzo beans, belongs to the Fabaceae family, with its seeds highly rich in protein. Owing to its versatility of sensory application and nut-like flavor in food, chickpea traditionally has been incorporated into a variety of culinary creations (Wallace et al. 2016). Chickpea prefers temperate and semi-arid regions and is said to be originated form Levant and ancient Egypt. With 64% of global chickpea production, India accounts for the highest chickpea production worldwide. The other major chickpea-producing countries include Pakistan, Turkey, Iran, Myanmar, Australia, Ethiopia, Canada, Mexico, and Iraq (Gaur et al. 2010). According to FAO, 14.2 tonnes of chickpeas were harvested worldwide in 2019 (Merga and Haji, 2019). The Kabuli type (light seeded) and the Desi type (dark seeded) are the two variety of chickpeas. Canada grows Kabuli type and desi chickpeas, in the southern parts of Alberta and

Saskatchewan. The exports of Canadian chickpea increased by 43% from 2019-2020 to 150,000 tonnes in 2020-2021. As the result of the larger supply, the carry out stocks were recorded to be 280 KT in 2020-2021. Due to increase in demand, the average price increased by 31% to $640/T for all chickpea types. By province, Saskatchewan is expected to account for 78% of the chickpea production, with 22% in Alberta in 2021-22.(Steve Lavergne and Fred Oleson 2021).

Although the variations in chickpea shape, seed size, and color, typically the seed is beaked and wrinkled or ribbed. The beak of the chickpea is the protruding seedling root tip (Shariati-Ievari 2013). In western culture, chickpea is consumed primarily through intake of hummus (Wallace et al. 2016).Chickpea significantly improves the fertility of soil by fixing nitrogen amount in atmosphere. 80% nitrogen requirement is achieved from symbiotic nitrogen fixation and chickpea can fix up to 140 kg N ha^{-1} from the air. A large amount of nitrogen residue is also left for the next crops adding plenty of organic matter for maintaining and improvement of health and fertility of soil. Chickpea can extract water from deeper layers in the soil due its deep tap root system and hence can withstand drought conditions (Gaur et al. 2010).

2.6 Environmental conditions of chickpea harvesting

In Prairies, the daily average temperature during harvest season ranges around 25 ℃. While harvesting and storage, chickpeas are subjected to a various range of temperatures. At temperatures below 10 ℃, microbial stability is naturally achieved as growth of microbes is minimized (Rani et al. 2013). Chickpea is a cool-season annual that requires approximately 110-120 days to reach harvesting stage. Its seeds are sowed in spring before May 25th in Saskatchewan and harvested in late September (Saskatchewan Pulse growers 2020). During this period, the daytime temperatures range between 21-29 ºC and night-time temperatures range between 18-21 ºC till harvest.

The moisture content at harvest affects seed quality and also helps to determine the threshing stage (Khatun et al. 2010). Seeds are prone to mechanical damage if harvesting is done at low moisture contents (<13%), whereas microflora infection occurs if they are harvested at high moisture (>18%). Normally, pulse crops are harvested at a moisture content of 20-22 %, followed by drying and storage at 12-14 % moisture content for 10 months preventing the risk of postharvest losses (Chidananda et al. 2014). In case of chickpea, combining can start when the seed moisture

content reaches to 18 %. At moisture contents less than 13 %, especially in Kabuli chickpea, the seeds begin to shrink from the seed coat and become highly susceptible to damage in handling (breakage). When possessing MC in the range of 14.1-16 % chickpeas are considered to be tough whereas they are damp at >16.0 % MC (Alberta pulse growers 2020).

2.7 Nutritional profile of chickpea

Pulses are a rich source of protein (19-22%), digestible and indigestible carbohydrates, polyphenols, and minerals. Albumins and globulins are the major proteins found in chickpeas along with smaller amounts of glutelins and prolamines (Saharan and Khetarpaul 1994). Chickpeas have dietary fiber of 17% and has low glycemic index (28%) making it a healthy food. National health and nutrition examination survey 2003-2010 was conducted to examine the association between chickpea/hummus consumption and nutrient intake in adults. The intake of many nutrients including dietary fiber, vitamin A, vitamin E, vitamin C, polyunsaturated fatty acids, potassium, folate, magnesium, and iron were higher in consumers of chickpea while comparing with non-consumers. Hummus has a higher Naturally Nutrient Rich (NNR) score in comparison to other dips. People consuming hummus have higher Healthy Eating Index 2005 (HEI-2005) scores (62.2 ± 1.3 v 51.9 ± 0.20). (Neil et al. 2014). Chickpea (raw or cooked) and hummus consists of dietary bio-actives such as phytic acid, sterols, tannins, carotenoids, and other polyphenols such as isoflavones, benefitting nutritional requirements of human consumption. The quality of protein in legumes, such as chickpea, is also improved by the heat treatment, which results in inactivation of many heat-liable anti-nutritional factors. This enhances the importance to persons following variations of plant-based diets and for vegans (Wallace et al. 2016).

2.8 Storage of Chickpea

2.8.1 Importance of storage studies

The average annual production of grains, oilseeds, and legumes in the world is 2.0 BT (Billion Tonnes). To meet the needs of the growing population and ensuring the availability of pulse crops over time, the development of safe storage conditions is imperative. Man-made ecological systems for bulk grain storage can undergo deterioration due to interaction among physical, biological, and chemical factors (Jayas and White 2003). On the other hand, to expand international trade, value chains are a foremost parameter to consider. There are two primary factors that contribute towards the complexity of the pulse sector value chain. Firstly, susceptibility of pulses to storage pests like bruchids. Hence, controlled temperature and humidity are required for long-term storage. The

absence of appropriate storage infrastructure can cause considerable losses. Secondly, milling of pulses is required for consumption, particularly in Asia. These factors lead to fluctuation in prices and a considerable price gap between producers and the consumers (Xia 2020).

In term of storage, the key factors for evaluation the life in crop storage are moisture content and storage temperature. Both the factors help is assessing the rate of the complex degradation reactions. Under optimal storage conditions, pulses are considered as edible for extended periods of time (Esther et al. 2015). However, in comparison to cereals, pulses are more prone to insects and microorganisms resulting in quality degradation (Mills and Woods 1994). Storability of seeds is affected by preharvest, harvest, and postharvest environments (Cassells and Caddick 2002). In general, higher range of temperature moisture content is stored commodities gives a short timeline for post-harvest operations. In case of wide range of moisture and temperature, determination of the allowable processing time before spoilage begins is essential. Safe storage guidelines are required for all crops at expected range of moisture contents and storage temperatures. These charts aid farmers in developing proper spoilage mitigation strategies before quality degradation and losses occur. Post-harvest treatments must be done within specific number of days after harvest to ensure quality of the crop (Nithya et al. 2011). Although, storage study data is available for various grains, pulses, and oilseeds, including desi chickpeas (Cassells and Caddick 2002) yet a comprehensive attempt to develop safe storage guidelines on Kabuli chickpeas has not been performed yet.

2.8.2 Variables affecting grain storage

Moisture content, temperature, and equilibrium relative humidity (ERH) are the potential variables involved in grain deterioration during storage (Copeland & Macdonald 2002.)

Moisture content is the amount of available water/moisture present in the seeds. Using potassium hydroxide to control the relative humidity in biological experiments has been practiced for a long time. Errors in controlling humidity when using salt solutions arise if absorption of water vapour takes place from damp materials or lose water through absorption of water vapor by enclosed materials within. Generally, at elevated temperatures, solutions tend to provide low range of humidity (Esther et al. 2015). Furthermore, if the solution is at a different temperature than the ambient air, the ERH will witness a deviation from the value expected. This leads to the relative humidity variation inside the storage bin causing the moisture content of samples to change (Sathya et al. 2008). Pockets of high moisture grain can lead to development of hotspots favoring

the growth of insects, microorganisms, and resulting in grain deterioration. Therefore, lower moisture crops can be stored for longer periods (Zomorodian et al. 2011). Sun (2014) reported that respiration rate is affected by moisture content of the seeds, water activity and RH in the storage chamber of the stored grain influencing the multiplication of molds and insects. Pixton et al. (1975) studied dry wheat (11.9% MC) and analyzed its quality changes over sixteen years of storage. At such low MC, wheat was not affected by any microorganisms for more than 10 years of storage, establishing that the main factor responsible for grain deterioration was high moisture content. Hence, high moisture grain pockets can act as a locus for infection by fungi and mites to multiply (Sun 2014).

Temperature also influences the quality of stored crops in storage. High temperature leads to increased respiration in stored grain resulting in fungal growth. Jayas and White (2003) stated that high moisture content and high temperature during storage accelerate the deterioration of grain. At high temperatures (≥ 30°C) an rise in the fatty acid value (FAV) and a decrease in germination was observed for pulse seeds (Chidananda et al. 2014). Temperature increases can cause a chain reaction starting with pest growth followed by inhibition of biological activity which could result in chemical oxidation. However, maintaining the temperature below 10 °C inhibits infection by storage fungi, except Penicillium (Singh 2017). Therefore, for safe and long-term storage of grains, maintaining lower temperatures (≤ 20°C) is suggested. Cassells and Caddick (2002) found that high relative humidity (≥ 70%) and high temperature (≥ 30°C) lead to deteriorating the seed quality during storage. The capacity of chickpea to uptake water is reduced when stored at high temperatures. Williams et al. (1983) showed the existence of a good correlation between time of cooking and hydration capacity. Late harvest can lead 'hard-to-cook' tendency in chickpeas. This condition leads to chickpeas not softening while being cooked and a force applied to achieve the cooking process was nominal 75% compression limit during conducted study. Storing the crop at high temperature and RH causes the 'hard-to-cook' phenomenon in pulses and is associated with the water absorbing capacity of the seed (Cassells and Caddick 2002)

Equilibrium moisture content (EMC) controls the water availability inside the stored crop's kernels. Hygroscopic materials absorb or lose moisture until they reach equilibrium with relative humidity. The relationship of EMC and ERH is reflected by sorption characteristics. The knowledge of EMC-ERH relationship also aids in optimization of storage and processing of

agricultural products and helps to determine the limit to which a crop will dry or rewet during storage (White et al. 1999). High RH results in undesirable chemical reactions and enzymatic activities adversely affecting the physical properties of grains and pulses (Bhat and Reddy 2017). Hence, low RH (≤ 50%) is favorable considering the germination index and the growth of seedling for storage of seeds for long-term (Pixton and Warburton 1971). For chickpeas, 70% RH at low moisture content is the threshold for mold growth when stored at the same temperature. Cassells and Caddick (2002) and Delouche (1988) suggested that for safe storage of chickpeas, the optimum RH is 60% and 50% at 20 °C and 30 °C, respectively. Multiplication of microflora occurs at RH above 75% for stored grain. More heat is produced due to microflora multiplication accelerating germination and deterioration of grains. For the safe storage of cereal grain and oilseed, 70% RH is considered 'safe' in equilibrium with the moisture content (Mills 1980).

2.9 Grain quality determination

2.9.1 Germination

Expressed in percentage, germination is an indication of living grain. Represented as transformation into the seedling, germination is the biological expression of physiological, biochemical, and morphological changes in a seed (Sheteiwy 2013; Sholberg 1975). In stored grains, seed germination is one the primary factor to evaluate the quality. Germination drop could be indicative of mold growth and grain spoilage among other factors such as mechanical damage (Rani et al. 2013). Moisture content, relative humidity, temperature, respiration, and light are the external factors that influence germination (Strenske et al. 2017) and the action of fungi and bacteria (Wagner et al. 2018). Viability of stored crops have been studies by many researchers. Kreyger (1972) concluded that at lower moisture contents, crops can be stored for a longer period after studying the effects of moisture and temperature on seed germination. With increasing storage parameters (moisture content, storage period and temperature), germination of black gram seeds have been shown to decrease (Esther et al. 2015). Also, crops at lower moisture content are prone to spoilage when stored at high temperatures (~40°C). Results were consistent with Christensen and Kauffman (1969) who reported injury and death of most types of grains with increasing temperature. Under optimal temperature and moisture content, storage longevity is positively associated with germination (Sheteiwy 2013). Hence, germination evaluation helps in indicating the safe and unsafe conditions for storage.

2.9.2 Free fatty acid Value

Oxidation of lipids and associated free radical oxidative stresses can cause seed deterioration and are associated with seed aging, thus influencing seed longevity (Crapiste et al. 1999). Many biochemical changes occur during storage leading to decrease in the nutritional value of stored products by altering carbohydrate, protein, lipid, and vitamin contents. Oxidative and hydrolytic changes occur in lipids producing off flavour or odor in the final product. Hence, for quality assessing of grain during storage, variation in lipid structure or constitution can be considered as an essential parameter. Microorganisms present in grains produce enzymes responsible for the hydrolysis of lipids. As the result of the breakdown of lipids during hydrolysis, FFA are produced. FFAs are produced at a faster rate if deterioration continues due to microbial growth. Thus, FFA is used as an index of deterioration by correlating it with the microorganisms present in the grain. Moisture content is positively correlated while temperature is negatively correlated with FFA. During storage, activity of lipolytic enzymes increases due to high relative as moisture content also increases (Wallace et al. 1983). Oxidation of lipids accelerates at high temperature and the process inhibits due to absence of oxygen (Singh 2017). Studies showed that at high temperature and high relative humidity, oilseeds (soybean) the oxidation of unsaturated fatty acid is also higher. (Stewart et al. 1980). Also, for optimal storage, high lipid seeds had requires lower moisture content and have lower thresholds for respiration (Priestley and Leopold 1983). Oxidation or hydrolysis leads to degradation of nutritional properties and alteration of lipid composition generating unpleasant tastes and odors. Furthermore, oil-soluble pigments are produced by the non-glyceride part of oil (FFA) that darkens its color. Hence, FAV is an important parameter to be considered for crop deterioration. In Rapeseed, germination drop and increase in FAV is an indicator of deterioration. (Mills 1980), wheat (Karunakaran et al. 2001; Nithya et al. 2011), rye (Sathya et al. 2008), and canola (Sathya et al. 2009), and pinto beans (Rani et al. 2013). FFA are produced due to hydrolytic reaction due to enzymatic secretions of micro-organisms in stored grain. These biochemical reactions have been observed to commence at accelerated pace due to an increase in moisture content in stored pinto beans (Rani et al. 2013). Characteristics odors are produced by these fatty acids. Hence, the FAV can be considered as the measure of quality deterioration crop during storage. The FAV is referred as KOH amount or mg KOH needed to neutralize the fatty acid in 100 g of dry seeds. The FAV values act as the index of deterioration in freshly harvested grain. (Sinha 1983).

2.9.3 Visible Mould

Fleurat-Lessard (2016) reported that when water activity (aw) in seeds exceeds a critical limit, fungal spoilage may occur. Maintaining large storage bin below the critical moisture limit during long term storage is not feasible hence, in stored commodities, an infection by seed-borne fungi occurs when the crop is stored under humid, temperate or hot climates. Under any storage conditions, growth of mold usually leads to discoloration of stored seed. A pocket of discolored seeds found in large bins is generally warmer than the other adjacent places, known as hotspots. Development of micro-organisms occurs in hotspots due to wet grain mainly at ≥ 0.65 aw (65% RH) (Sauer 1988). Rapid growth of microorganisms reflects the visible mold growth and helps to determine the spoilage. As microorganisms grow, heat is produced leading to more hotspots through a self-accelerating process inside the storage bin. Esther et al. (2015) found that high storage temperature and moisture content promotes the fungal growth and the appearance of visible mold in black grams. Fungal infection in stored grains alters the grain quality by declining the germination in seed, and degrading the quality of nutrition. Hence, visible mold appearance by external microscopic observation and the off-odor from crop was considered as a spoilage index for evaluation the safe index for the beans and field peas, grains, and oilseeds (Mills and Woods 1994). For seeds with higher moisture content, visible mold can appear sooner irrespective of the storage temperature (Sathya et al. 2008). Therefore, visible mould is a good subjective measure of grain quality (Bhat and Reddy 2017). Mould spoilage can be controlled when: i) moisture level is maintained below the critical limit of fungal growth; ii) respiration of storage fungi is monitored by maintaining water activity and temperature changes during the storage period; iii) grain's bulk moistening trends are reduced through physical intervention; iv) mycotoxin contamination is inhibited by applying physical treatments (ozone, grain peeling or abrasion); bio-competitive strains of fungi or bacteria are implemented for the prevention of mycotoxigenic fungi development in grain bulks (Fleurat-Lessard 2016).

2.9.3 Protein Analysis

Besides carbohydrates and fats, proteins are one of the macronutrients vital for life and required for the proper functioning of cells, tissues, organs, and body systems as a whole. Derived from both animal and plant tissue, food proteins are non-toxic and can be digested by the human body to provide nutrition in the form of amino acids (Rodrigues et al. 2012). The protein content of pulses is approximately 21-25%, however, they possess essential amino acids such as cystine,

methionine, and tryptophan in limited amount (Singh 2017). Pulse variety, germination, environment, and application of fertilizers are responsible for variation in protein content. Pulses protein is almost double as compared with the protein in cereals. Two major fractions of pulse proteins have been classified as albumin and globulin with the latter comprising 35-72% of total protein and the remaining being primarily albumins. Specialized subcellular compartments contains proteins knowns as protein bodies. Protein bodies are often devoid of catalytic activity. After germination, proteins are mobilized supporting early growth and development of seedling, referred as storage proteins. These molecules enhance the nutritional quality of the seeds and functional properties of the derived food. (Argos et al. 1985). A negative correlation with digestibility and solubility of protein was observed during the long term storage of black beans in adverse conditions (Molina et al. 1976). Enzymatic and non-enzymatic reactions resulting this defect lead to the 'hard-to-cook' phenomenon in pulses (Reyes-Moreno et al. 2000). Studies show that denaturation in protein and pectin in-solubilization results in seed inability to soften during cooking. Inter as well intra-cellular water availability was also controlled due to this affect (Hincks and Stanley 1987). Jawad et al. (2013) found that the phytate, protein, and tannin contents decreased as storage time increased at 29 ℃ and correlated those changes to an increase in the cooking time of common beans (Phaseolus vulgaris L.). Studies on cowpeas showed that due to hydrolysis of lipids, FFA are formed. This lowers the pH of beans during storage and further leads to in-solubilization, reversible denaturation, and decreased extractability of proteins (Hentges et al. 1991). (Mitchell and Beadles 1949)Mitchell and Beadles found that when storing the cereal grains under conditions of low moisture content (than the critical moisture content) to prevent insect infestation and growth of fungi, the nutritive value of the proteins of cereal grains does not alter due respiration of seeds over long periods.

2.10 Safe storage guidelines for different crops

In well managed facilities and developed countries, postharvest grain losses can vary from 1-2% where as 20-50% in less developed countries with poorly managed storage system (Jayas 2012), There are different reasons for farmers, traders and governments for storing crops apart from the profit enhancement from these commodities. Farming systems, Trading enterprise and government policies all involves storge and have contribution to other activities or objectives within these broader contexts. Grains are stored due to: difference is location of consumption and production, seasonal production and year round consumption, difference in location of processing and

production, to cope with emergency situation such as famine and seed kept for the next sowing period (Jayas 2012). Delivering the optimal condition of crop storage aids farmer to attend the next crop for operations and other farm activities. The development of safe storage guidelines helps the growers to develop automated systems of storing crops in large bins. Crop storage with automated systems like temperature sensing systems, drying and aeration controllers will help the farmers, equipment dealers, and researchers for designing a feasible environment of storage. It is important to design the safe storage guidelines for the new crop to avoid the spoilage, wastage, and economic losses faced in the handling crop. The storage guidelines could also be included in the web application for the farmers to assess the data easily and take decisions for the long term and safe storage of the stored seeds.

Safe storage guidelines are developed for various crops by numerous researchers. The main parameters studied in developing safe storage guidelines are moisture content, temperature, and ERH. The procedures to set-up up controlled environmental conditions and quality evaluation can be followed easily and aid valuable knowledge to other researchers in this area. The trends of some studies are given below.

Sathya et al. (2009) developed the storage guidelines for canola based on different moisture content and temperature ranges. It was concluded that, canola possessing moisture content of <10% stored at <20 ℃ is safe for 15 weeks, whereas the seeds at 12.5 and 15.0% MC stored at 25 ℃ are suggested to be dried within a week to avoid spoilage (Figure 2).

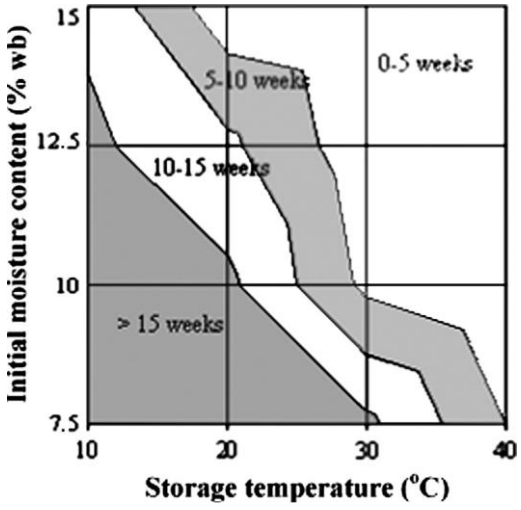

Figure 2 Estimated safe storage life of Canola (Sathya et al. 2009)

Rye was also studied for designing the safe storage guidelines at different moisture and temperature ranges by Sathya et al. (2008). The authors stated that rye stored at ≤20 ℃ with ≤12.5% moisture content would be safe for at least 15 weeks, whereas rye stored 40 ℃ at possessing ≥15% have to be completely dried within less than a week before storing for long term (Figure 3).

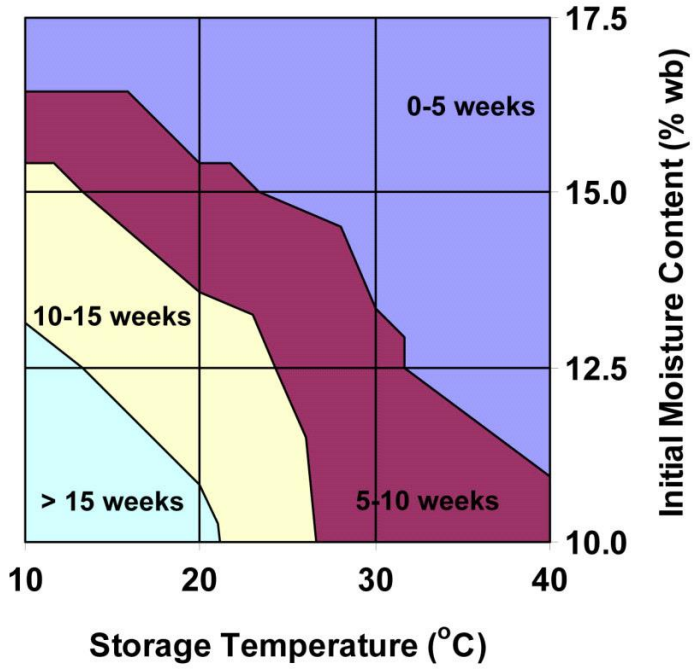

Figure 3 Estimated safe storage life of rye (Sathya et al. 2008)

Figure 4 shows the storage guidelines for duram wheat developed by Nithya et al. (2011). Findings showed that germination declined with rise in in moisture content, temperature, and storage period. High moisture samples (17, 18, 19 and 20%) stored at high temperatures had visible mold in them. FAV of the samples showed positive correlation with storage time and moisture content.

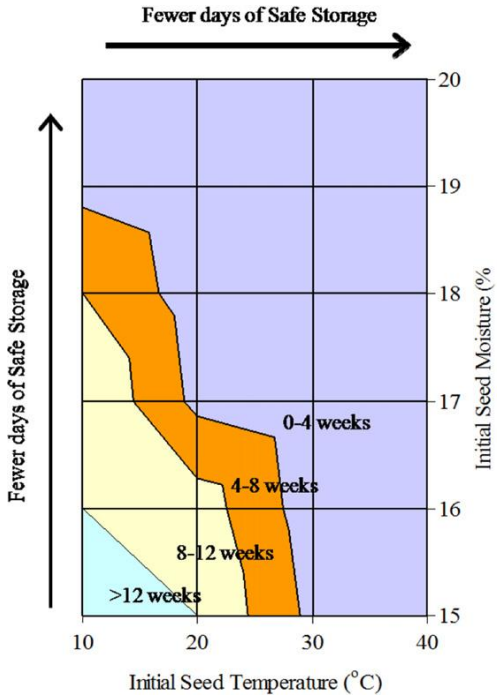

Figure 4 Estimated safe storage life of durum wheat (Nithya et al. 2011)

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Rani et al. (2013) carried out storage studies on pinto (Figure 5). It was concluded by Rani et al. (2013) that beans stored at lower moisture content (12 and 14% w.b.) and lower temperatures (10 and 20 ℃) maintained appreciable seed germination, seed coat color, and microbial stability for 16 weeks. Whereas, at higher moisture content of 16, 18, and 20% w.b. if there is need to store for long term, beans must be dried before 8, 5, and 3 weeks of storage, respectively.

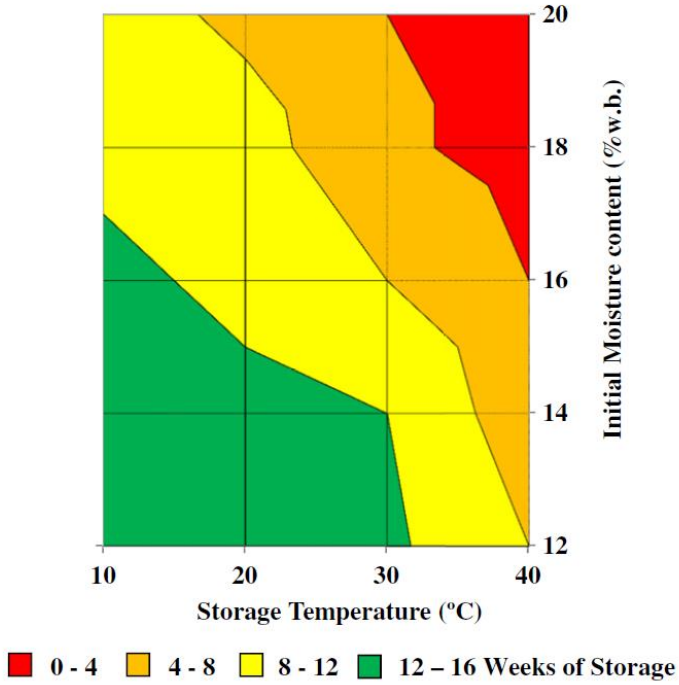

Figure 5 Estimated safe storage life of pinto beans (Rani et al. 2013).

3 MATERIALS AND METHODS

3.1 Selection of moisture and temperature regimes

Kabuli chickpeas (300 kg) were obtained from Reisner Farms in Limerick, Saskatchewan at a moisture content of 11 % (w.b.). The moisture content was determined by placing three replicates of 10 g each in a hot air oven at 103 ℃ for 72 h (ASABE 2008). Equation 1 was used to determine the moisture content.

$$Moisture\ Content\ = \frac{m_f - m_i}{m_f} \times 100 \qquad (1)$$

Where, m_f depicts final mass of the chickpeas, m_i= initial mass of the sample

To simulate environmental conditions of the Prairie-grown chickpeas during harvest and storage, temperatures of 10, 20, and 30 ℃ were selected for conducting the storage study (Esther et al. 2015). Further, four saturated salt solutions, namely, $Mg(NO_3)_2$, $NaNO_2$, NaCl, and KNO_3 were utilized to produce 54, 65, 75 and 94% RH in storage, respectively (Jian et al. 2019, Miles and Glynn 2017). The equilibrium moisture content of chickpeas for storage at 54, 65, 75 and 94% RH was calculated as 9, 11, 13, and 15 %, respectively based on the equilibrium moisture content-relative humidity (EMC-RH) equation using modified Henderson model (Armstrong et al. 2017) and harvesting moisture content of chickpeas. The RH in the environmental chambers was maintained from 60-70 % throughout the storage period. The conditioning of samples was done to the required initial moisture contents of 9, 11, 13, and 15 % by calculating the amount of water to be added using Equation 2:

$$X = \frac{m(M_f - M_i)}{1 - M_f} \times 100 \qquad (2)$$

where, X is the amount of water to be added (L); *m* depicts the mass of grain (kg); M_f is the final (desired) moisture content; M_i represents the initial moisture content.

3.2 Experimental setup

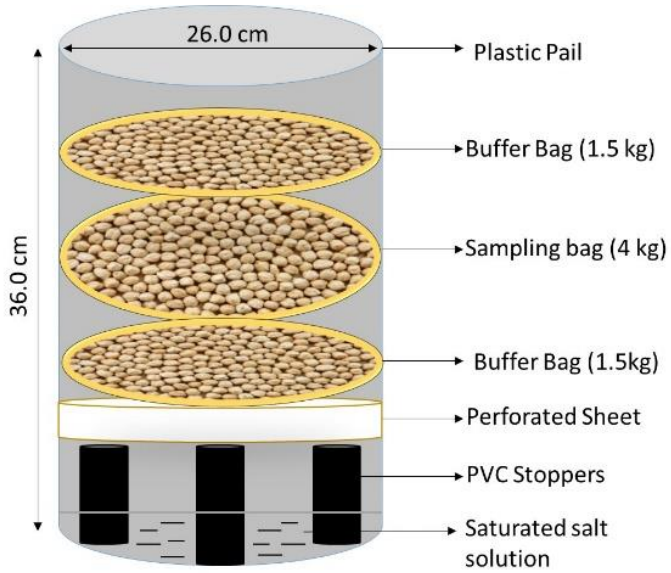

Figure 6 Schematic diagram of the experimental arrangement of chickpea samples in 20 L pail

Plastic pails (diameter 26.0 cm and height 36.0 cm) were used for storing the chickpeas. In each pail, three PVC pipes (about 8 cm in diameter and 10 cm in height) were used for providing support to the mesh plate. Beneath the mesh plate, each pail contained 2 L of saturated salt solutions. Every pail contained 4 kg sampling mesh bags with a mesh size of <2.5 mm in the middle whereas two buffer sample bags (1.5 kg each) were placed on the top and bottom of the sampling bag. Buffer sample bags contributed towards prevention of moisture loss from the sampling bag. The pails were loosely covered by a lid enabling air circulation. Three environmental chambers (Conviron CMP3244, Controlled Environments Ltd., Winnipeg, MB) were used to maintain 10, 20, and 30 °C (±2 °C) at a relative humidity of 60-70 %. Three replicates were used for each moisture and temperature combination. Each environmental chamber had 12 pails. Samples were acquired on weekly basis after thoroughly mixing the sampling bag. About 150 g (per week) of sample was taken out at regular intervals for 16 weeks to analyze moisture content, germination, FAV and protein content. Every week, the chickpea samples were thoroughly mixed to avoid anaerobiosis and the accumulation of gaseous metabolites from seeds and fungi (Mills and Woods 1994).

3.3 Seed quality assessment

The quality parameters procedures were similar to earlier studies (Sathya et al. 2008; Rajarammanna et al. 2010; Nithya et al. 2011; Rani et al. 2013; Jian et al. 2019)which identified the factors causing deterioration, helping in the mitigation of losses leading to development of safe storage guidelines. Each parameter was evaluated in triplicates from every temperature and RH combination.

3.3.1 Germination

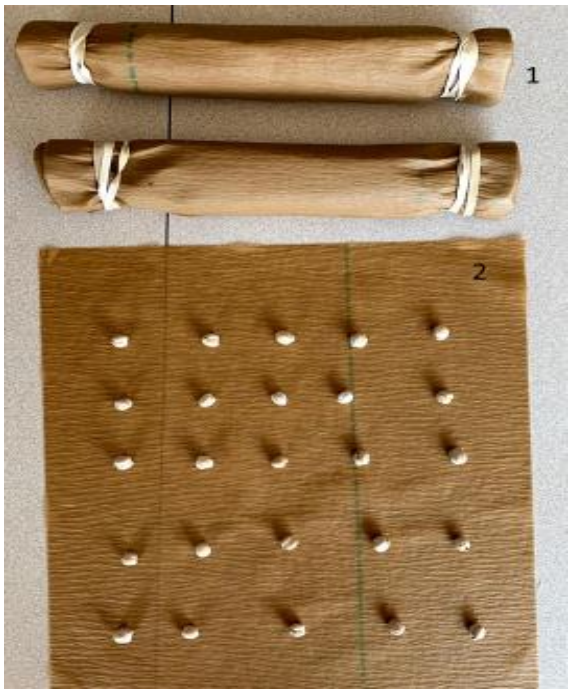

Figure 2 Germination by between paper method (1) Chickpeas rolled in germination paper (2) Placing of 25 chickpeas for germination test

For the determination of germination percentage, between paper method was used (Gold 2009; CFIA 2011). The germination paper was moistened with distilled water and 25 seeds were placed in 5 rows with equal spacing. The germination percentage was determined after counting the number of seeds germinated after 8 d of incubation

3.3.2 Moisture conten.

Every week, the moisture content was determined by placing three replicates of (10 g) from each temperature and RH combination in a hot air oven for 72 h at 103 ℃ (±2 ℃) (ASABE 2008). The

moisture content of the sample was calculated and expressed on a percentage (w.b) as shown in Equation 1.

3.3.3 Visible mould

Every week, samples were visually inspected for the determination of visible mould. Although subjective, this method is good enough in indicating advanced deterioration.

3.3.4 Fatty acid Value (FAV)

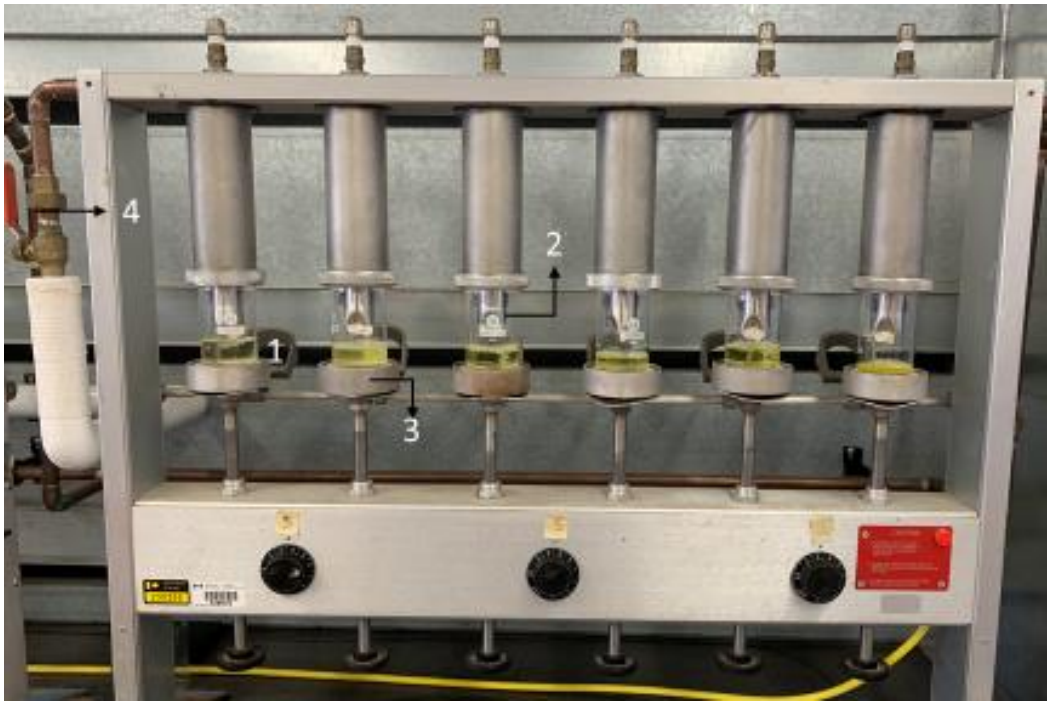

Figure 8 Goldfisch fat extractor for FAV analysis (1) Thermal beakers (2) Sample holder with 5 g of ground chickpeas in filter paper (3) Heating platform (4) Continuous supply of water

The Goldfisch fat extractor method was used for the determination of FFAcontent (American Association of Cereal Chemists procedure, 1962). The samples (100 g) were grounded using the stein mill (M-2, Fred Stein Laboratories, Inc, Atchinson, KS) running each batch for 3 minutes after bone-drying the samples in a hot air oven at 103 °C for 72 h. For each replicate ground samples (5 g) were weighed and rolled in Whatman No. 5 filter paper and placed inside sample holder tubes for fat extraction (Goldfisch Fat Extractor, Laboratory Construction Co, Kansas City, MO) with 30 mL of petroleum ether solvent in beakers. The oil was extracted in a duration of 6 h.

Continuous monitoring is required to check if the solvents evaporate completely or if there is a leak. Add more solvent simultaneously if it seems to evaporate quickly. Afterward, solvent was evaporated, and the oil was separated by heating it again. Further, 25 mL of TAP solution (50% toluene and 50% ethanol with phenolphthalein indicator) was added to the oil. A KOH solution of known normality (0.01152 Eq/L) was used for titration until the appearance of a pale pink color. Calculation of fat acidity value (FAV) is defined as milligrams of KOH required to neutralize the fatty acids in 100 grams of dry grain as shown in equation 3.

$$\text{FAV} = \frac{(ml\ KOH - blank)(mg\ KOH/\ ml\ solution) \times 100}{weight\ of\ sample} \qquad (3)$$

3.3.5 Protein analysis

Figure 9 LECO apparatus (1) Helium supply (2) Oxygen supply (3) compressed air supply (4) sample holder

Protein content was analyzed using LECO apparatus (FP-628 Protein Analyzer, LECO Corporation, St. Joseph, MI, USA)). Nitrogen analysis is used for determination of the total protein. Until specified temperature and pressure is reached, ballast tank collects the combusted samples with oxygen and the gases containing nitrogen oxides. Helium act as a carrier and an

aliquot of combustion gas having nitrogen oxides reduced to nitrogen. Water and carbon dioxide are removed by passing it through a tube containing magnesium perchlorate and sodium hydroxide on a silicate. A thermal conductivity detector measures the nitrogen using helium as a reference. Nitrogen is then converted to protein using a conversion factor of 6.25 (Nestares et al. 1996).

Homogeneously ground dried chickpeas were used for protein analysis. The flour samples (0.2 g) were wrapped in tin foils. Before analysing the chickpea samples, five blanks and 3 EDTA standards were run until three consecutive values with a Relative Standard Deviation of 0.2% or less were obtained.

3.4 Statistical analysis

Analysis of variance (ANOVA) of a three-factorial design model (4 moisture contents × 3 storage temperatures × 16 weeks) was done to learn the effect on dependant variables (moisture content, germination, FAV, protein content). Quantitative variables were compared pairwise using least significance difference and Tukey's test in SPSS software (Version 25). The difference was tested at 95% confidence interval within each level under each variable.

4 RESULTS AND DISCUSSION

4.1 Moisture content

Table 1 depicts the ANOVA statistics for the moisture content of chickpeas. It was observed that the moisture content of the stored chickpeas changed significantly with the storage period, temperature and ERH. However, the combined interaction of storage variables did not affect the moisture content significantly (Table 1). Combined interaction was studied to see the impact of two or more independent variable on dependent variable. Similar observations were recorded by Majid et al. (2014) for chickpeas in which water acitvity signifinactly affected the moisture content during the storage. A rise in moisture cotent with increasing temperature and storage period was also reported by

Table 1 ANOVA statistics for moisture content of chickpeas

Storage Factors	DF	F	Sig. (P Value)
Temperature	2	23.337	0.000
Storage Period	1	510.746	0.000
ERH	3	376.573	0.000
Temperature*Storage Period	2	18.519	0.000
Temperature*ERH	6	1.534	0.187
Storage period*ERH	3	39.528	0.000
Temperature*Storage Period*ERH	6	0.594	0.733

The moisture content of chickpeas at 10 °C increased gradually at four different RH conditions. In case of the samples stored at 94% RH, the moisture content increased by 1% while the samples stored at 75% and 65% RH conditions depicted a 2% gain in moisture content. The highest increase in moisture was observed for samples possessing an initial moisture content of 9% (54% RH) at 10 °C which reached to 12.5% at the end of the 16-week storage period. Figure 7 shows the change

in moisture content of chickpea samples stored at all temperatures at initial moisture contents of 9, 11, 13, and 15%, respectively.

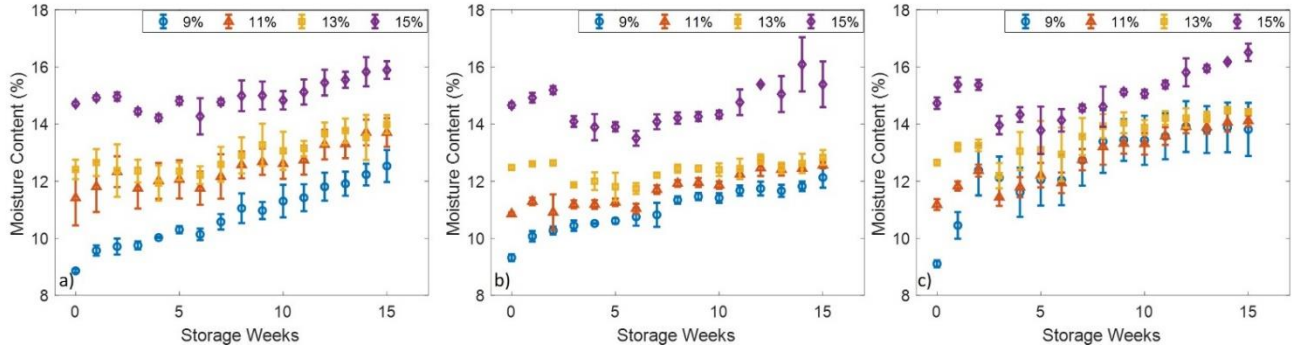

Figure 10 Changes in moisture content (% w.b.) (± standard deviation) of stored chickpeas at (a) 10 ℃, (b) 20 ℃, (c) 30 ℃ with respect to storage period

In case of chickpea samples stored at 20 ℃, the moisture content reached around 13% for 60, 75, and 54% RH conditions whereas the moisture content did not depict any statistically significant changes for samples at 94% RH. Results showed that the increase in moisture content for samples kept at 30 ℃ was higher as compared to the lower temperatures. In this case, the samples stored at RH of 54, 65, and 75% reached a moisture content of 14% whereas the samples in 94% RH attained a moisture content value of 16.5% at the end of the 16-week storage period. Similar trends were reported by Chidananda et al. (2014) where higher temperature lead to higher respiration in the seeds correlating positively with change in moisture content. Thus, the moisture content of stored grain correlates positively with storage temperature and storage period of the grain. Similar trends were reported by Rani et al. (2013), Nithya et al. (2011) and Sravanthi et al. (2013) for pinto beans, durum wheat, and red lentils, respectively. This indicates that the initial moisture content of chickpeas was lower than the equilibrium moisture content. Similar findings of an increase in moisture content due to water activity were observed by Majid et al. (2014), in which chickpea cultivars were stored for 60 days at different water activity values of 0.5, 0.6, and 0.7. The results also correlated with those of black gram samples where differences in the storage and ambient temperatures was the cause for deviation of the equilibrium humidity resulting in a change in the moisture content of the samples (Esther et al. 2015).

4.2 Germination

Table 2 shows the ANOVA statistics for the germination. Kabuli chickpeas depicted a statistically significant change in germination with the increase in storage period, ERH, and temperature (p=0.000). The interaction among all the storage variables also depicted a statistically significant effect on the seed germination portraying a negative correlation. Similar findings were reported for hemp by Jian et al. (2019) where the RH had a statistically significant effect on seed germination. Higher temperature and higher moisture content can promote fungal growth reducing the seed viability during storage. Moreover, significant variation in seed germination with respect to temperature and storage period was also observed in black grams and pinto beans (Esther et al. 2015; Rani et al. 2013).

Table 2 ANOVA statistics for germination of chickpeas

Storage Factors	DF	F	Sig. (P-Value)
Temperature	2	354.737	0.000
Storage Period	1	1731.390	0.000
ERH	3	83.921	0.000
Temperature*Storage Period	2	352.890	0.000
Temperature*ERH	6	32.354	0.000
Storage period*ERH	3	60.520	0.000
Temperature*Storage Period*ERH	6	32.494	0.000

Initial germination of the chickpea seeds was 97%. At 10 ℃, throughout storage, the percentage of germination stayed over 85% for the samples kept in RH of 54%, 65%, and 75% at moisture levels 9, 11, and 13%, respectively. Similar findings were reported for pinto beans kept at low moisture contents of 12 and 14% (Rani et al. 2013). However, at high RH (94%), germination decreased to less than 60%. The samples kept at 20 ℃ at RH conditions of 54, 65, and 75% showed a gradual drop in germination whereas the seeds kept at 94% RH, experienced a substantial decrease in germination after the 11^{th} week of storage. The seed viability reached zero after 5^{th}, 10^{th} , 12^{th} and 13^{th} week of storage for samples kept at 54, 65, 75 and 94% RH at 30 ℃.

Germination percent reached to zero after the 5 weeks of storage for samples placed in RH of 94%. For 54, 65, and 75% RH, the percentage germination reduced to zero after the 13, 12, and 10 weeks of storage, respectively. Thus, it validates that germination of high moisture samples decreases quickly during storage above 20℃. Also, high temperature has a negative correlation with germination. ANOVA results in table 2 depicted that germination of chickpea samples was significantly affected by temperature, moisture content, and storage period (α=0.05). The results were in relation to the previous storage studies for wheat (Karunakaran et al.,2001; Nithya et al. 2011), canola (Sathya et al. 2009) and pinto beans (Rani et al. 2013) .

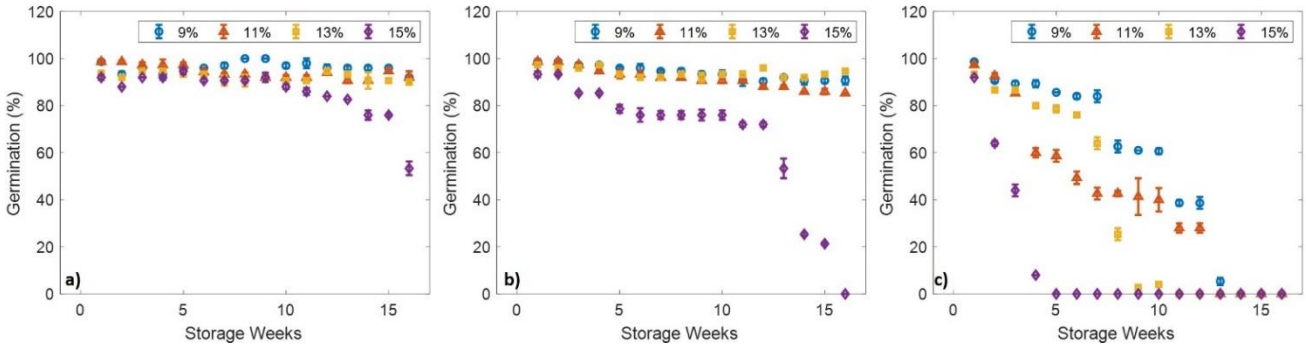

Figure 11 Changes in chickpea germination percentage (± standard deviation) of stored chickpeas at (a) 10 ℃, (b) 20 ℃, and (c) 30 ℃ at different storage intervals

4.3 Visible Mould

Visible mold was observed for samples possessing high moisture content stored at high temperature. The samples at 10 and 20 ℃ were not infected with fungi and the mold appeared only at the high moisture contents (15% and 13%) at 30 ℃. It started to appear when the germination started declining (Rani et al. 2013).

Table 3 Time of first appearance of visible mould on chickpea (week) and the germination % on the same week

Moisture Content		9	11	13	15
Storge Temperature (℃)	Replicate	Week, %	Week, %	Week, %	Week, %
10	1	-	-	-	-
	2	-	-	-	-

	3	-	-	-	-
20	1	-	-	-	13,72
	2	-	-	-	13,40
	2	-	-	-	13,48
30	1	13,72	12,28	10,4	4,8
	2	13,32	12,20	10,4	4,8
	3	13,12	12,36	10,4	4,8

4.4 Protein

Table 4 ANOVA statistics for protein content

Storage Factors	DF	F	Sig. (P-Value)
Temperature	2	1.612	0.81
Storage Period	1	4.063	0.049
ERH	3	3.370	0.026
Temperature*Storage Period	2	0.949	0.394
Temperature*ERH	6	0.658	0.684
Storage period*ERH	3	0.776	0.513
Temperature*Storage Period*ERH	6	0.581	0.743

The ANOVA statistics for the protein content are depicted in table 5. For the stored chickpeas, the change in protein content was not statistically significant with respect to all the storage variables (temperature, storage period, and ERH). The result was consistent with Majid et al. (2014) where protein content of chickpeas changed insignificantly with the combined as well as independent affect of water activity and storage time. The initial protein content of the conditioned chickpea samples was determined to be 19.5% ± 0.1%, 19.82 ± 0.1, 19.53 ± 0.3, and 19.7% ± 0.4% at 9, 11, 13, and 15% moisture contents, respectively. For the crude protein content, there were no statistically significant differences over the storage period for samples stored at different temperatures and RH conditions. The result was consistent with Majid et al. 2014 where protein

content was changed insignificantly with the combined as well and independent affect of water activity and storage time. Protein bodies as embedded in starch particles remain intact and are not hydrolyzed by mold. This is in agreement with (Bulbula and Urga 2018) which indicated that even after germination of chickpeas, the decrease in protein content was not statistically significant.

4.5 Free fatty acid value

Table 5 ANOVA statistics for FAV

Storage Factors	DF	F	Sig. (P-Value)
Temperature	2	33.325	0.000
Storage Period	1	114.787	0.000
ERH	3	13.975	0.000
Temperature*Storage Period	2	25.354	0.000
Temperature*ERH	6	2.139	0.066
Storage period*ERH	3	3.600	0.020
Temperature*Storage Period*ERH	6	2.083	0.73

Table 4 represents the ANOVA statistics for FAV. The FAV depicted a statistically significant variation with storage period, temperature, and ERH. However, the combined effect of storage variables on FAV was insignificant though there was a positive correlation between storage variables and FAV. The results were consistent with those of pinto beans where the samples stored at high temperature and moisture content lost their viability due to a significant increase in their FAV (Rani et al. 2013). Storage period and moisture content showed a significant affect on FAV for black gram samples (Esther et al. 2015). The significant variation due to RH was also consistent with the storage studies of hemp (Jian et al. 2019). Lipid oxidation and hydrolytic reactions due to the presence of microorganisms can cause the production of FFAs. Higher values of FFA are an indication of the deterioration of grains. Figure 8 shows the changes in FFAat different moisture content and temperatures. The values of FFA correlated positively with moisture content and temperature. It was observed that the lowest moisture had the lowest value of FFAs. These results correlated with the study of Chidananda et al. (2014), in which the FAV of chickpea at 12%

moisture content was 8.1 mg of KOH/100 g and at 20% moisture content was 14.5 mg of KOH/100 g. Similarly, for samples at 9% moisture content, the FAV were 2.56, 3.67, and 3.82 mg of KOH/100 g at 10, 20, and 30 ℃, respectively, for samples from the 1st week of storage. At the 16th week of storage, FAV were determined to be 6.38, 6.39, and 23.25 mg of KOH/100 g for samples possessing 9% moisture content at 10, 20, and 30 ℃, respectively. Samples stored at 65% RH witnessed an increase in FAV from 5.17 to 8.1 mg of KOH/100 g at 10 ℃ and 3.87 to 8.1 mg of KOH/100g at 20 ℃ during the 16-week storage period. Almost similar trends were observed for samples stored in 75% RH at 10 and 20 ℃. The samples at 15% moisture content and RH of 94% depicted an increase in FAV from 6.02 to 11.07 mg of KOH/100g at 10 ℃ and 5.15 to 13.42 mg of KOH/100g at 20 ℃ throughout storage. A significant increase in FAV was observed for all the samples stored at 30 ℃. The samples stored at RH of 65, 75, and 94% portrayed an increase of almost four folds with initial FAV of 5.12, 5.15, and 7.23 mg of KOH/100 g during the 1st week to 17.55, 19.96, and 31.60 mg of KOH/100 g, respectively, at the end of the 16-week storage study. The observed relation between FAV and moisture content was consistent with that of black gram (Esther et al. 2015). Another study by Srivastava and Vasishtha (2013) showed that after soaking, the lipid content of chickpea decreases. A decrease in lipid content is directly correlated with an increase in FAV due to the increase in moisture content as a result of soaking. Furthermore, the effect of moisture content, temperature, and storage period on the FAV content concurred with the storage studies on wheat (Karunakaran et al. 2001; Nithya et al. 2011), rye (Rajarammanna et al. 2010), and pinto beans (Rani et al. 2013). Due to proliferation of mould at high moisture contents and temperatures, more fatty acids are produced in commodities (Nithya et al. 2011; Esther et al. 2015).

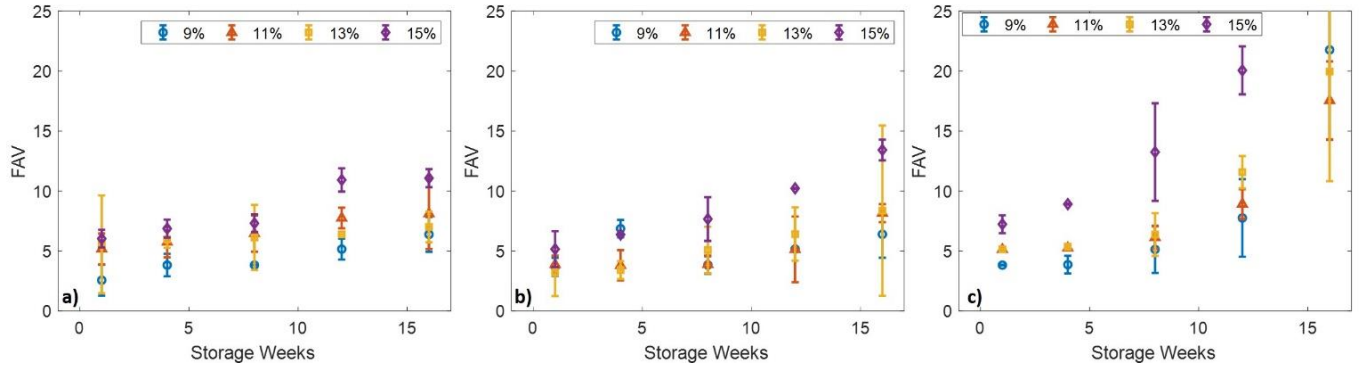

Figure 12 Changes in FAV (mg of KOH/100 g of dry chickpeas) (± standard deviation) of stored chickpeas at (a) 10 ℃, (b) 20 ℃, and (c) 30 ℃ at different storage intervals

4.6 Estimated Storage Life

The safe storage guideline chart for chickpeas based on decrease in germination and appearance of visible mould at different temperatures and moisture levels is shown in Figure 5. When the germination reaches less than 80%, the crop is not considered safe to store. Considering a given species of mould may produce large amounts of FAV, it is not considered a measure of deterioration. Chickpeas are safe to store at 10 and 20 ℃ at 9, 11, and 13% moisture content for storage periods of ≥16 weeks. It is not advisable to store chickpeas at high temperatures approaching ~30 ℃. Under exceptional circumstances, if they are to be stored at such high temperature, the moisture content should be reduced to 11% before 6^{th} week of storage. For optimal and long-term storage of chickpeas, the temperature of storage must be below 20 ℃. It was also suggested by Delouche (1988) and Cassells and Caddick (2002) that chickpeas are safe to store at RH of 50 and 60% at 20 and 30 ℃, respectively. Similarly, the safe moisture content to store chickpeas is recommended to be 12.5 and 9.5% at 20 and 30 ℃, respectively (Cassells and Caddick 2002).

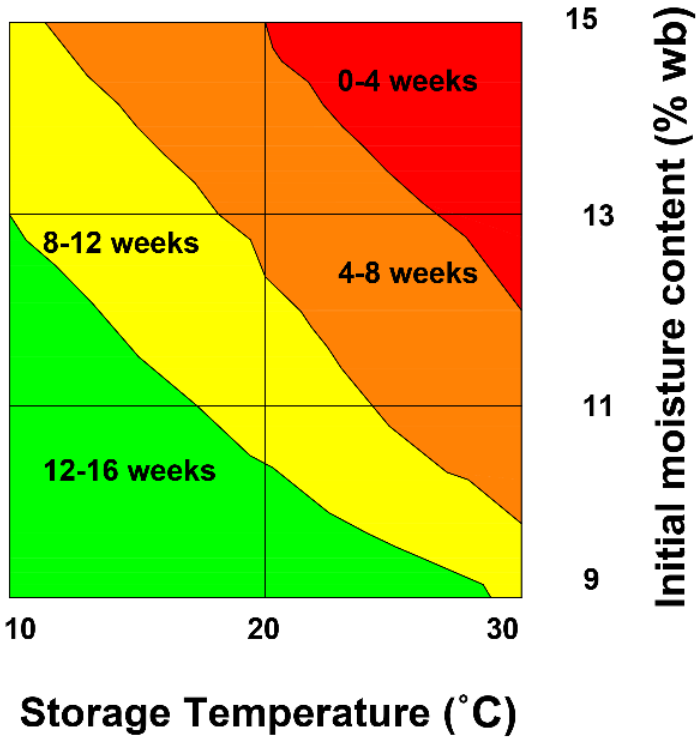

Figure 13 Recommended safe storage guideline chart for Kabuli chickpeas

5 CONCLUSIONS

The moisture content, storage temperature, and storage period showed a significant effect on the germination and FAV of chickpea. The storage variables depicted a positive correlation with FAV and a negative correlation with germination. For the samples stored at moisture content of 9, 11, and 13% and 10 and 20 ℃ temperature, germination remianed 80% of higher throughout the 16-week period of storage. In contrast, the values of germination decreased significantly for samples at 15% moisture content stored at 20 and 30 ℃. The samples lost their viability at 15% moisture content during the 15^{th} week of storage at 20 ℃. Germination for samples stored at high temperature (i.e., 30 ℃) reduced to zero at all moisture contents during storage. The relation between FAV and germination was observed to be inversely proportional. The FAV showed a positive correlation for all temperatures and moisture contents over the period of storage. FAV increased four folds for the samples stored at 30 ℃. Appearance of visible mould was observed for chickpeas stored at 30 ℃ over time for all moisture contents. For long term storage, it is recommended to store chickpeas below 13% moisture content and below 20 ℃ to maintain the quality of seed and its viability.

6 RECOMMENDATIONS FOR FUTURE RESEARCH

- Duration of storage period can be extended to evaluate the further changes in the quality of chickpeas for the long term
- Changes in the crop characteristics and quality can be analysed with the application of non-destructive optical methods
- Developing safe storage standards for other legume and cereal crops
- Other parameters such as change is cooking quality, nutritional changes, anti-nutritional properties, etc. can be analysed based on storage
- Developing an web application for the farmers using the guidelines for safe storage as a model

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APPENDIX
APPENDIX A MOISTURE CONTENT

Table A1 Moisture content of chickpea seed stored at 10℃

Storage Weeks	RH	Moisture Content (Temperature 10℃)			Mean	SD
		R1	R2	R3		
1	94	14.71	14.78	14.65	14.71	0.06
2	94	14.87	15.01	14.87	14.92	0.08
4	94	14.32	14.46	14.53	14.44	0.10
6	94	14.79	14.95	14.68	14.81	0.13
8	94	14.70	14.71	14.90	14.77	0.11
10	94	15.30	14.42	15.26	14.99	0.49
12	94	15.31	14.62	15.45	15.12	0.44
14	94	15.57	15.25	15.82	15.55	0.28
16	94	15.98	15.54	16.15	15.89	0.31
1	75	12.47	12.05	12.72	12.41	0.34
2	75	12.13	12.75	13.06	12.65	0.47
4	75	11.90	12.67	12.47	12.35	0.4
6	75	12.04	12.67	12.35	12.35	0.31
8	75	13.28	12.12	12.36	12.59	0.61
10	75	12.79	14.13	12.90	13.27	0.74
12	75	12.86	13.41	13.14	13.14	0.27
14	75	13.32	14.16	13.83	13.77	0.42
16	75	13.71	14.35	13.93	13.99	0.32
1	65	10.77	10.95	12.51	11.41	0.95
2	65	11.27	11.30	12.84	11.80	0.89
4	65	11.90	12.67	12.47	11.75	0.71
6	65	12.84	11.71	11.63	12.06	0.67
8	65	13.02	11.97	11.49	12.16	0.78
10	65	13.21	12.47	12.36	12.68	0.46
12	65	13.32	12.57	12.34	12.74	0.51
14	65	13.88	12.92	13.10	13.30	0.50
16	65	14.27	13.48	13.33	13.70	0.50

1	54	8.80	8.88	8.92	8.86	0.06
2	54	9.47	9.46	9.79	9.57	0.18
4	54	9.66	9.91	9.65	9.74	0.14
6	54	10.42	10.33	10.15	10.30	0.13
8	54	10.59	10.29	10.83	10.57	0.27
10	54	11.04	10.64	11.23	10.97	0.30
12	54	11.80	10.90	11.58	11.42	0.47
14	54	12.21	11.43	12.10	11.91	0.42
16	54	12.89	11.88	12.82	12.53	0.56

R1, R2 and R3 =Replicate 1,2 and 3 respectively. All are same in these appendixes

Table A2 Moisture content of chickpea seed stored at 20℃

Storage Weeks	RH	Moisture Content (Temperature 20℃)			Mean	SD
		R1	R2	R3		
1	94	14.59	14.62	14.77	14.66	0.09
2	94	15.29	15.25	15.03	14.92	0.17
4	94	15.55	15.71	14.41	14.09	0.19
6	94	13.27	13.43	13.79	13.90	0.16
8	94	14.12	14.05	14.44	14.08	0.26
10	94	14.20	14.32	14.48	14.26	0.16
12	94	15.36	15.42	15.38	14.76	0.45
14	94	15.36	15.32	15.50	15.05	0.63
16	94	14.48	16.00	15.69	15.39	0.80
1	75	12.57	12.44	12.42	12.48	0.08
2	75	12.56	12.62	12.74	12.60	0.02
4	75	11.98	11.68	12.32	11.87	0.02
6	75	11.66	11.59	11.96	11.80	0.49
8	75	12.41	12.32	12.60	12.21	0.08
10	75	12.37	12.19	12.63	12.44	0.10
12	75	12.59	12.80	12.92	12.44	0.35
14	75	12.32	12.67	12.39	12.46	0.18
16	75	12.64	13.12	12.71	12.83	0.26
1	65	10.92	10.82	10.81	10.85	0.06

2	65	11.41	10.18	11.12	11.29	0.02
4	65	11.34	11.17	11.02	11.18	0.14
6	65	10.94	11.24	10.94	11.26	0.13
8	65	11.78	12.00	11.99	11.70	0.15
10	65	11.73	12.00	11.86	11.95	0.16
12	65	12.34	12.78	12.24	12.24	0.16
14	65	12.32	12.65	12.24	12.40	0.21
16	65	12.44	12.63	12.61	12.56	0.10
1	54	9.42	9.36	9.18	9.32	0.12
2	54	10.15	10.23	10.43	10.07	0.18
4	54	10.53	10.52	10.50	10.44	0.18
6	54	11.11	10.54	10.60	10.61	0.09
8	54	11.41	11.18	11.41	10.82	0.42
10	54	11.58	11.43	11.22	11.46	0.13
12	54	11.84	11.92	11.46	11.67	0.18
14	54	11.56	11.91	11.51	11.66	0.21
16	54	11.81	12.06	12.53	12.13	0.36

Table A3 Moisture content of chickpea seed stored at 30℃

Storage Weeks	RH	Moisture Content (Temperature 30℃)			Mean	SD
		R1	R2	R3		
1	94	14.96	14.61	14.16	14.73	0.20
2	94	15.66	15.31	15.17	15.38	0.25
4	94	13.75	14.33	13.79	13.96	0.32
6	94	14.49	13.86	13.98	13.78	0.83
8	94	14.53	14.45	14.70	14.56	0.12
10	94	15.23	15.03	15.10	15.12	0.10
12	94	15.26	15.34	15.54	15.38	0.14
14	94	16.08	15.94	15.83	15.95	0.12
16	94	16.54	16.18	16.79	16.51	0.30
1	75	12.74	12.67	12.55	12.65	0.09
2	75	13.26	13.29	13.29	13.19	0.15

4	75	12.52	12.38	11.68	12.19	0.44
6	75	14.26	12.46	12.59	13.10	1.00
8	75	14.31	13.29	13.08	13.56	0.65
10	75	14.45	13.95	13.71	14.04	0.37
12	75	14.35	14.15	13.86	14.12	0.24
14	75	14.44	14.24	14.07	14.25	0.18
16	75	14.43	14.48	14.39	14.43	0.04
1	65	11.32	10.95	11.26	11.18	0.19
2	65	11.70	12.02	11.77	11.83	0.16
4	65	11.40	11.76	11.15	11.44	0.30
6	65	11.72	12.58	12.32	12.21	0.43
8	65	12.57	13.18	12.51	12.75	0.37
10	65	13.19	13.73	13.04	13.32	0.36
12	65	13.46	13.93	13.34	13.58	0.30
14	65	13.72	14.16	13.79	13.89	0.23
16	65	13.91	14.10	14.30	14.11	0.19
1	54	9.00	9.24	9.06	9.10	0.12
2	54	9.93	10.84	10.57	10.45	0.46
4	54	11.80	11.59	12.96	12.12	0.74
6	54	11.13	12.95	12.05	12.05	0.90
8	54	11.76	13.52	12.92	12.73	0.89
10	54	12.64	14.10	13.58	13.44	0.73
12	54	12.70	13.72	14.29	13.57	0.80
14	54	12.86	14.40	14.14	13.80	0.82
16	54	12.76	14.54	14.14	13.81	0.93

APPENDIX B: GERMINATION DATA

Table B1. Germination of chickpea seed stored at 10°C

Storage Weeks	RH	Germination (Temperature 10°C)			Mean	SD
		R1	R2	R3		
1	94	88	96	92	92	1
2	94	88	88	88	88	0

4	94	92	96	88	92	1
6	94	88	92	92	90.66	0.5
8	94	88	92	92	90.66	0.5
10	94	84	80	88	84	1
12	94	84	84	84	84	0
14	94	76	84	68	76	2
16	94	40	60	60	53.33	2.8
1	75	88	92	100	93.33	1.5
2	75	92	92	92	92	0
4	75	88	88	88	88	0
6	75	88	84	84	85.33	0.5
8	75	80	92	100	90.66	2.5
10	75	88	88	88	88	0
12	75	92	100	100	97.33	1.15
14	75	72	88	100	86.66	3.5
16	75	44	84	84	70.66	2.5
1	65	96	92	100	96	1
2	65	92	96	92	93.33	0.5
4	65	84	100	100	94.66	2.3
6	65	88	96	92	92	1
8	65	96	100	96	97.33	0.5
10	65	88	92	96	92	1
12	65	100	100	96	98.66	0.5
14	65	88	88	96	90.66	1.1
16	65	60	52	64	58.66	2.5
1	54	100	100	96	98.66	0.5
2	54	96	96	88	93.33	1.1
4	54	100	88	88	92	1.7
6	54	92	92	92	92	0
8	54	100	100	100	100	0
10	54	96	96	96	96	0
12	54	100	96	88	94.66	1.5
14	54	92	96	100	96	1

16	54	80	96	100	92	2.6

Table B2 Germination of chickpea seed stored at 20℃

Storage Weeks	RH	Germination (Temperature 20℃)			Mean	SD
		R1	R2	R3		
1	94	96	88	96	93.33	0.5
2	94	88	88	84	86.66	0.5
4	94	84	84	88	85.33	0.5
6	94	72	92	72	78.66	1.7
8	94	72	84	72	76	1.1
10	94	76	84	68	76	1.7
12	94	68	72	76	72	1.1
14	94	24	24	28	25.33	1.7
16	94	0	0	0	0	2.5
1	75	96	92	100	96	1
2	75	92	92	96	93.33	0.5
4	75	96	88	96	93.33	0.5
6	75	96	92	92	93.33	0.5
8	75	96	96	96	96	0
10	75	100	92	100	97.33	1.1
12	75	100	96	96	97.33	0.5
14	75	92	96	88	92	1
16	75	96	96	92	94.66	0.5
1	65	92	100	100	97.33	1.1
2	65	96	100	100	98.66	0.5
4	65	96	96	92	94.66	0.5
6	65	84	84	84	84	0
8	65	84	84	80	82.66	0.5
10	65	84	84	92	86.66	1.1
12	65	76	80	76	77.33	0.5
14	65	80	80	80	80	1
16	65	72	72	72	72	0

1	54	96	96	100	97.33	1.1
2	54	92	88	92	90.66	0.5
4	54	88	92	92	90.66	0.5
6	54	88	92	92	90.66	2.8
8	54	92	92	100	94.66	1.7
10	54	100	100	88	96	2
12	54	92	100	100	97.33	1
14	54	88	100	100	96	0.5
16	54	92	80	100	90.66	0

Table BC Germination of chickpea seed stored at 30℃

Storage Weeks	RH	Germination (Temperature 30℃)			Mean	SD
		R1	R2	R3		
1	94	92	92	92	92	0.5
2	94	60	68	64	64	0.5
4	94	8	8	8	8	1.5
6	94	0	0	0	0	1.1
8	94	0	0	0	0	5.1
10	94	0	0	0	0	4.5
12	94	0	0	0	0	7.6
14	94	0	0	0	0	0
16	94	0	0	0	0	0
1	75	92	96	92	93.33	0.5
2	75	88	84	88	86.66	0.5
4	75	80	76	84	80	1
6	75	80	72	76	76	1
8	75	0	36	40	25.33	5.5
10	75	4	4	4	4	0
12	75	0	0	0	0	0
14	75	0	0	0	0	0
16	75	0	0	0	0	0
1	65	96	96	100	97.33	0.5

2	65	88	80	88	85.33	1.1
4	65	60	68	52	60	2
6	65	48	28	44	40	2.6
8	65	44	44	36	41.33	1.1
10	65	40	20	60	40	5
12	65	36	20	28	28	2
14	65	0	0	0	0	0
16	65	0	0	0	0	0
1	54	100	100	96	98.66	0
2	54	84	84	88	85.33	1
4	54	84	88	96	89.33	0
6	54	84	92	92	89.33	0
8	54	56	84	44	61.33	0
10	54	80	44	64	62.66	0
12	54	72	32	12	38.66	0
14	54	0	0	0	0	0
16	54	0	0	0	0	0

APPENDIX C: GERMINATION DATA

Table C1 FAV of chickpea seed stored at 20℃

Storage Weeks	RH	FAV (Temperature 20℃)			Mean	SD
		R1	R2	R3		
1	94	5.14	7.74	7.74	5.15	1.5
4	94	5.14	5.15	6.76	6.86	0.007
8	94	6.37	8.95	7.15	7.66	1.8
12	94	10.19	10.25	10.25	10.22	0.04
16	94	12.81	14.03	11.81	13.42	0.86
1	75	3.86	6.45	2.57	3.21	1.9
4	75	2.53	3.82	3.84	3.40	0.7
8	75	2.53	3.83	5.08	5.08	1.9
12	75	5.15	5.13	8.99	6.41	2.2
16	75	9.00	7.73	10.62	8.39	7.1

1	65	5.16	3.87	5.16	3.87	0.7
4	65	2.56	3.8	1.28	3.8	1.2
8	65	3.87	3.87	2.58	3.87	0.7
12	65	5.13	9.00	5.13	5.12	2.7
16	65	7.72	8.89	7.64	8.11	0.7
1	54	3.863	2.56	2.55	3.67	0.7
4	54	1.29	2.54	2.56	3.86	0.7
8	54	2.57	3.83	3.84	3.83	0.7
12	54	5.09	5.09	5.16	5.16	0.05
16	54	7.67	5.11	5.11	6.39	1.9

Table C2 FAV of chickpea seed stored at 10℃

Storage Weeks	RH	FAV (Temperature 10℃)			Mean	SD
		R1	R2	R3		
1	94	6.45	5.17	6.45	6.023	0.7
4	94	7.73	6.43	6.44	6.86	0.7
8	94	7.74	7.73	6.45	7.30	0.7
12	94	10.24	11.61	10.24	10.92	0.9
16	94	11.58	11.44	10.21	11.07	0.7
1	75	3.87	10.2	3.87	5.55	4.07
4	75	5.16	6.4	6.37	5.97	0.7
8	75	5.15	8.99	5.15	6.12	2.7
12	75	6.38	6.39	6.38	6.38	0.01
16	75	6.35	5.09	7.65	7.00	1.28
1	65	5.17	6.44	5.14	5.17	1.28
4	65	6.37	6.4	5.14	5.74	1.2
8	65	3.82	5.15	5.15	6.45	1.5
12	65	6.38	5.15	5.15	7.74	0.8
16	65	5.09	5.11	5.09	8.1	2.9
1	54	3.87	3.87	2.55	2.56	1.29
4	54	3.82	5.15	5.15	3.82	0.9
8	54	3.82	3.82	3.82	3.82	0

12	54	6.38	5.15	5.15	5.15	0.86
16	54	5.09	5.11	5.08	6.38	1.4

Table C3 FAV of chickpea seed stored at 30℃

Storage Weeks	RH	FAV (Temperature 30℃)			Mean	SD
		R1	R2	R3		
1	94	7.71	6.38	7.62	7.23	0.7
4	94	8.93	8.86	8.92	8.98	0.3
8	94	11.55	10.30	17.89	13.24	4.06
12	94	17.86	20.58	21.76	20.06	2.00
16	94	29.53	30.53	34.75	31.60	2.77
1	75	5.15	5.16	5.16	5.15	0.005
4	75	3.86	5.15	6.15	5.35	0.02
8	75	5.09	3.58	6.15	6.37	1.7
12	75	10.23	11.56	12.92	11.57	1.3
16	75	16.78	23.14	15.58	19.96	9.1
1	65	5.15	5.15	5.14	5.12	0.02
4	65	5.15	5.17	5.21	5.25	0.02
8	65	3.83	5.13	5.13	6.12	0.9
12	65	7.67	10.15	8.92	8.91	1.2
16	65	14.09	20.55	18.01	17.55	3.2
1	54	3.81	3.86	3.81	3.82	0.02
4	54	3.83	2.57	2.54	3.86	0.7
8	54	3.83	5.13	5.13	5.13	1.9
12	54	5.09	11.53	7.05	7.75	3.2
16	54	9.00	20.3	23.25	21.77	7.5