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Comparison of deterioration of rye under different storage regimes

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Abstract. Deterioration of stored rye samples were studied at different moisture contents and temperatures. Germination, appearance of visible and invisible microflora, and fatty acid values (FAV) were determined for samples at 10.0, 12.5, 15.0 and 17.5% moisture content (wet mass basis) stored at 10, 20, 30 and 40^{o}C for 16 weeks. During the experiment, results of deterioration at constant moisture content were compared with previously reported results for deterioration under declining moisture content. Germination rate was almost the same for all the moisture content samples stored at 10^{o}C for both cases, but a significant decrease was observed at other temperatures with high moisture content. Fatty acid values remained similar in both the cases stored at 10 and 20^{o}C, whereas at 30 and 40^{o}C, fatty acid values of the rye samples which maintained the moisture content were high. Visible mould appeared early in the samples whose moisture content was maintained and increased with an increase in temperature and moisture content during the experiment. *Penicillium spp*. and *Aspergillus glaucus* were the predominant fungal species present in both the cases throughout the study. Significant difference in rate of deterioration was observed during the study for both the cases.

Keywords: *Canola, rye, temperature, moisture content, safe storage period.*

Introduction

Rye (*Secale cereale* L.) is the second major cereal crop used in the baking industries next to wheat. Rye is usually harvested in August or September in Canada. Since rye has no virtual dormancy period, it is swathed at 45% moisture content. If not it may lead to pre-harvest sprouting (Hartman 1999). In addition to this, weather plays a vital role in the production and harvesting of the crop. For example, in 1996 due to wet autumn weather only 50% of the wheat crop was harvested (Anonymous 1997). Though rye is harvested at high moisture, it is dried to 22% moisture content before threshing and the threshed rye has to be dried to 14% or less before storing for longer period of time.

Storage is a critical stage of grain production. Storage can be defined as the interim phase occurring on the farm, at different collection points serving a number of farms and in terminal elevators and plants (Anderson et al. 1943). Safe storage of grain is holding the grain for long time without any significant quality and quantity changes. In Canada around 80% of harvested grain is stored on-farm. During such storage there are many factors that can affect the quality of grain such as temperature at which the grain is stored, moisture content, seed maturity and condition, storage time, inter granular gas composition, insects, microorganisms, mites, moulds, rodents, birds, dockage, granary structure and geographical location (Jayas 1995). Of the above factors, storage temperature and moisture content of the stored grain are the two main physical factors that have to be monitored continuously. This is because growth and multiplication of all the living organisms in grain depends on these two factors (Jayas 1995). When these two factors exceed the safety levels, visible and invisible mould starts to grow thereby deteriorating the grain quality (Bottomley et al. 1952).

During storage the quality of grain can be monitored by following a few important parameters such as seed germination, fungal growth, fatty acid value (FAV), gluten quality and nutritive changes (Muir 2001). Among all these factors, germination is considered the primary factor in assessing the quality of grain (Pomeranz 1992). Bottomley et al. (1952) reported that as the moisture content increased, viability of the grain decreased. They also reported that at higher moisture levels (31.9% in equilibrium with 100% relative humidity) viability reached zero soon after 12 days of storage.

Presence of microflora is a quick indication of mould growth in stored grain. One of the simplest methods of enumerating the mould growth is by placing 25 grain kernels on a filter paper saturated with 7.5% sodium chloride aqueous solution for one week (Wallace and Sinha 1962; Sinha 1983; Friday et al. 1986). Among the field fungi *Alternaria* spp. are predominant while *Penicillium* spp. and *Aspergillus* spp. are dominant storage fungi (Wallace and Sinha 1962; Christensen and Kaufmann 1969).

Another important parameter to be considered in monitoring the deterioration of grain is the fatty acid value (FAV). It is done by solvent extraction by extracting fatty acids from 5 g of dried representative sample and titrating with potassium hydroxide solution (Schroth 1996). Fat acid value has positive correlation with moisture content of the sample (Bottomley et al. 1952).

Many studies were conducted to determine the quality of stored grain by using these factors. The quality changes in rapeseed (Mills and Sinha 1980), wheat (Wallace et al. 1983), canola meal (White and Jayas 1989), flax seed (White and Jayas 1991), wild rice and rice (White and Jayas 1996), hull-less and hulled oats and barley (White et al. 1999a), solin (White et al 1999b), maize (Zia-Ur-Rehman et al. 2002), rice, wheat and maize (Zia-Ur-Rehman 2006), rye and canola (Sathya 2006, Sathya et al. 2008), durum wheat (Nithya 2008) have been studied during storage. In many cases there were declines in moisture content when the samples were stored at high temperatures for long period. Hence an experimental setup was designed in such a way

that the initial moisture content of the rye samples were retained throughout the study even at elevated temperature and moisture content. The results of deterioration of rye samples obtained from this study were compared with the results of rye samples that showed a decline in moisture content during the course of the experiment (Sathya et al. 2008).

Materials and Methods

Remington variety of fall rye (400 kg) was obtained from Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg. Samples were obtained at 14% (wet mass basis) moisture content and were reconditioned to 10.0, 12.5, 15.0, 17.5% moisture content. Since it is not advisable to dry the harvested rye artificially before it reaches 20% moisture content (Hartman 1999), all the rye samples will have moisture content below 20% before undergoing any post harvest treatment. Also the straight grade for rye is 14% moisture content (Anonymous 2006). Hence a moisture range of 10.0-17.5% was selected for this study. The moisture contents (±0.2%) above 14% required for the study were achieved by adding a calculated quantity of distilled water. The conditioned samples were kept in plastic bags in a fridge at 5^{o}C ± 2^{o}C for 72 h. To ensure the uniform moisture distribution, the grain in the plastic bag was mixed thoroughly every 3 h for 3 days and the final moisture was determined by a hot air oven. The samples were stored in a freezer (-5^{o}C ± 2^{o}C) until used for the experiments. To lower the moisture content below 14% to the required level, the grain samples were spread and dried in the ambient air inside a closed room for several hours and the moisture content was monitored regularly until it reached the desired level.

The entire experiment was carried out in environmental chambers (Models E15 and C1010, CONVIRON, Controlled Environments Limited, Winnipeg, MB and CRELAB, Climatic Research Equipment, Model: WHL3-610M, Winnipeg, MB). All selected chambers were maintained at four different temperatures of 10, 20, 30 and 40^{o}C (±2^{o}C) with relative humidity (RH) of around 70±5%. The temperature range of 10-40^{o}C was selected based on the range of temperatures the grain would be exposed to during and after harvest.

Equilibrium Relative Humidity (ERH) of 60, 75, 85 and 90% RH were maintained for 10.0, 12.5, 15.0, 17.5% moisture content samples using potassium hydroxide (KOH) solutions of respective specific gravities of 1.285, 1.211, 1.147 and 1.108 (Solomon 1951).

Design of experimental setup

To maintain the moisture content of the stored grain sample, 2 L of KOH of known specific gravity was placed at the bottom of plastic pails with a capacity of 15 L. Conditioned grain samples were placed in mesh bags holding 3 and 5 kg. Sampling for 16 weeks was from 5 kg mesh bag while other two 3 kg bags on the top and bottom acted as buffer. It was hypothesized that the buffer samples placed above and below the 5 kg sampling bag with same moisture content would prevent the moisture loss. A lid was loosely placed on the top of the pail. For each temperature and moisture content combination, three replicates were done. Sub-samples for quality analysis were taken every week by thoroughly mixing the 5 kg rye samples. Sampling was carried out continuously for 16 weeks.

Quality assessment parameters

Measuring the moisture content was an extremely important factor, because this was a comparative study that dealt with results of deterioration at constant moisture content with previously reported results for deterioration under declining moisture content of Sathya (2006). Moisture content, in triplicate, was usually expressed on a wet mass basis. It was measured by drying 10 g of sample in a hot air oven at 130^{o}C for 16 h (ASAE 2003).

Among the grain quality assessment parameters, germination was measured first, because it is a good indicator of grain quality and further analysis may not be necessary once the seed looses its viability. Once the seed losses its viability it is hard to store them and deterioration progresses rapidly. Germination was measured from the sub-samples collected every week by placing 25 seeds on Whatman no. 3 filter paper saturated with 5.5 ml of distilled water in a 90 mm diameter petri dish. The dishes were piled one above the other in a stand and were covered with polythene wrap for the first 4 days at room temperature (22±1^{o}C). After that the wrap was removed by exposing the dishes to ambient air for 3 days and the number of seeds germinated was counted (Wallace and Sinha 1962).

Fatty acid values (FAV), were also an important parameter and they were measured for the sub-samples collected biweekly. The sub-samples were dried in a hot air oven at 130^{o}C for 16 h and a known amount of dried grain was ground to extract FAV using a Goldfisch fat extractor followed by KOH titration (Schroth 1996).

Invisible mould was also enumerated from the sub-samples collected biweekly. Twenty five seeds from the sample were displayed on a Whatman no.3 filter paper saturated with 5.5 ml of 7.5% aqueous sodium chloride (NaCl) solution in a 90 mm diameter petri dish. The dishes were piled one above the other in a stand and were covered with polythene wrap for the first 4 days at room temperature (22±1^{o}C). After that the wrap was removed by exposing the dishes to ambient air for 3 days and the number of seeds showing the growth of microfloral species was identified and enumerated using a dissection microscope (Mills et al. 1978). Appearance of visible mould was verified by visually inspecting the stored sample in plastic pail.

Statistical analysis

In this study, a three factorial design model (16 weeks × 4 temperatures × 4 moisture contents) of analysis of variance (ANOVA) was used to analyze the effects of duration of experiment, temperature, moisture content on germination and fatty acid values. By least significant method, the changes occurred in germination and FAV were analyzed with 95% confidence level.

Results and Discussion

The results obtained from the current study which maintained the moisture content throughout the period of 16 weeks was taken as case 1 and the results of the previously reported study where the moisture was allowed to decline was taken as case 2 (Sathya et al. 2008). Moisture content, germination, FAV, visible and invisible mold data for both the cases were compared and are discussed below.

Moisture content

Moisture content of the sample is an important parameter when storing the grain for long periods. The moisture was maintained for the entire study period even at elevated temperatures (30 and 40^{o}C) which were not possible in many reported studies (Zia-Ur-Rehman 2006, Sathya et al. 2008). The experiment was designed in such a way that potassium hydroxide (KOH) solutions of different specific gravities were placed under the respective samples in pails to maintain the required relative humidity. A decline in moisture content was further prevented by having buffer samples above and below the reference sample.

Figure 1 shows the comparative changes in moisture content (% wb) with respect to storage period. At 10^{o}C the samples showed a slight increase in moisture content while moisture content of samples stored at 20^{o}C remained almost the same throughout the study in case 1. This is similar to what was observed in the samples of case 2. But at higher temperatures there were significant decline in moisture content of the samples for case 2 (Sathya et al. 2008). The samples stored at 30^{o}C without buffer and 2 L of KOH at 15.0 and 17.5% moisture content

showed a steady decline in moisture and reached 12.5% moisture at the end of 16 weeks. But for the samples stored with buffer samples and 2 litre of KOH, the moisture content remained almost constant.

For the rye samples stored at 40^{o}C, there was a drastic difference in moisture content between the samples stored with and without buffer samples. In case 2, moisture content of the 10.0 and 12.5% moisture samples reached around 5% moisture content at the end of the study while the high moisture content samples at 15.0 and 17.5% reached around 8 and 10% moisture content as early as the 5th and 6th week. But the samples stored with buffer samples maintained almost the same moisture throughout 16 weeks of study.

Germination

Germination of the rye samples stored at 10, 20, 30 and 40^{o}C for a period of 16 weeks is shown (Fig. 2). Seed germination for rye samples in case 1 at the start of the experiment was 92%. This remained above 80% for the samples stored at 10^{o}C with 10.0, 12.5 and 15.0% moisture content throughout the experiment. In case 2, the germination of 80% was maintained only for the samples stored at 10^{o}C with 10.0 and 12.5% moisture content. In both the cases germination decreased with an increase in storage temperature irrespective of moisture content.

Rye samples stored with 15.0 and 17.5 % moisture content at 40^{o}C reached 0% germination as early as in 3rd and 4th weeks respectively for case 1, compared to 0% germination at 5th and 6th weeks for case 2 (Sathya et al. 2008). Overall, the germination of the samples in case 1 showed more decrease when compared to the samples stored at the same moisture contents and temperatures in case 2 (Sathya et al. 2008). This may be because in case 2 (Sathya et al. 2008), samples stored at higher temperatures showed decline in moisture from the second week, while in case 1 there was no such decline in moisture. Since the rate of deterioration of grain is higher when stored at higher moisture, the germination percentage of case 1 which maintained the initial moisture content even at higher temperatures, showed more decrease in germination than case 2 (Sathya et al. 2008). All the factors, moisture content, storage temperature and storage period had significant effect on the germination of rye samples (α=0.05).

Fatty acid value

Due to oxidative or hydrolytic biochemical changes occurring in the grain stored over a period of time, there will be nutritive loss in stored grain (Pomeranz 1992). The free fatty acids (FFA) are formed by the hydrolysis reaction caused by the enzymatic secretions of the associated micro-organisms in stored grain. Since high moisture grains favor mold growth, the changes in FAV occurring in the high moisture grain is also high. Therefore, fatty acid value (FAV) is an index to monitor the quality of stored grain (Christensen and Kaufmann 1969). As there is no absolute value to correlate FAV with deterioration of grain, relative change in FAV is used to associate the deterioration (Sinha 1983).

The FAV values for both the cases are given in Fig. 3. At 10 and 20^{o}C the moisture content of all the samples were maintained throughout the study for both the cases. Therefore, there was not much difference in trend of fatty acid values produced in both the cases. For the samples stored at 30 and 40^{o}C, there was significant difference in fatty acid values for both the cases. For the samples of case 1, the study showed increased fatty acid values for all moisture contents when compared to the amount of fatty acid values of the samples in case 2. This is because mould thrives at high moisture grain and the samples that showed steady decline in moisture may not have supported mould growth eventually ending in lower fatty acid values. The same trend was observed in the samples stored at 40^{o}C. Moisture content, storage temperature and storage period had significant effect on the fatty acid values of rye.

Microflora

Fungi developed in stored grain are responsible for the deterioration of grain, hence it is important to verify the microfloral growth to quantify the amount of deterioration. Irrespective of temperature and moisture content, visible mould appeared in all 17.5% moisture content samples for both cases.

For the samples with higher moisture content of 17.5%, visible mould appeared in the first week of storage at all temperatures except 10^{o}C in both the cases (Table 1). As the mould started to grow it produced a musty odour which was associated with increased fungal growth and decreased germination (Wallace et al. 1983).

During the initial storage time *Penicillium spp*. were found predominantly in all samples in both the cases. At 10 and 20^{o}C, *Penicillium spp*. were predominant while *A. glaucus* replaced them at higher storage temperatures (30 and 40^{o}C). Bacteria appeared rarely at cooler temperatures for samples of case 1. *Alternaria* was abundant in samples stored at 10^{o}C and scarcely at higher temperatures while there was no trace of *Alternaria* in the samples which had declining moisture content. However the presence of *Alternaria* cannot be related with storage aspects as it is a field fungus which does not multiply in storage.

Hormodendrum was found less frequently in almost all samples but it was not detected in any of the samples of case 1. *Aspergillus ochraceus* increased with moisture content and storage period in both the cases. At 30^{o}C, samples showed increased growth of *A. glaucus* in both the cases. But for the samples of case 1, for the samples stored at 40^{o}C, lesser growth of microflora was found when compared to the samples of case 2. This result supports the conclusions of Nithya (2008) and Christensen and Kaufmann (1969). Other fungi such as *A. niger, Fusarium* and *Rhizopus* were found rarely in some samples but they could not be compared and related.

Storage fungi pose a huge threat to stored grain because its growth and metabolic activity lead to decreased germination rate and contamination of the sample. Fungi also produce mycotoxins which are dangerous to animal and human health (Nithya 2008). To prevent these fungal growths the storage temperature should be below 20^{o}C and the moisture content should be below 15.0%. Below this temperature and moisture fungi cannot grow (Abramson et al. 1990).

Conclusions

By comparing the rye samples of case 1 and 2, when stored with 10.0, 12.5, 15.0 and 17.5% initial moisture content at 10, 20, 30 and 40^{o}C for 16 wk the following conclusions were made.

At 10 and 20^{o}C the samples in both the cases maintained the moisture content but at elevated temperatures of 30 and 40^{o}C due to the absence of buffer samples there was decline in moisture in case 2.

Germination decreased quickly for the samples that maintained the initial moisture content because moulds, which affect germination greatly, need a certain amount of water activity for growth and presence of moisture throughout the study helped them thrive in these conditions eventually leading to quicker deterioration of rye samples.

FAV was found high in the samples of case 1 at higher temperatures when compared to the samples of case 2. Also visible mould appeared earlier in the samples of case 1. At elevated temperature of 40^{o}C, microfloral growth was much less in case 1 when compared to rye samples of case 2 in later part of the storage.

The above differences may be due to the presence of moisture content throughout the experiment in case 1 while it was not so in case 2 (Sathya et al. 2008). So it can be concluded that only the rye samples with ≤12.5% moisture content stored at ≤ 20^{o}C would be safe for >15

weeks irrespective of maintaining or declining moisture content. By retaining the moisture in samples the seed lost viability and started to deteriorate earlier than the rye samples in case 2.

Acknowledgements

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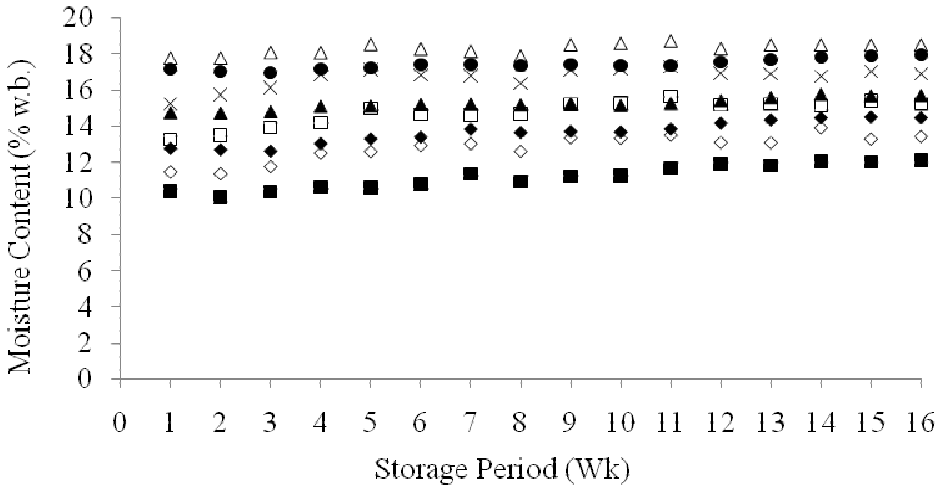
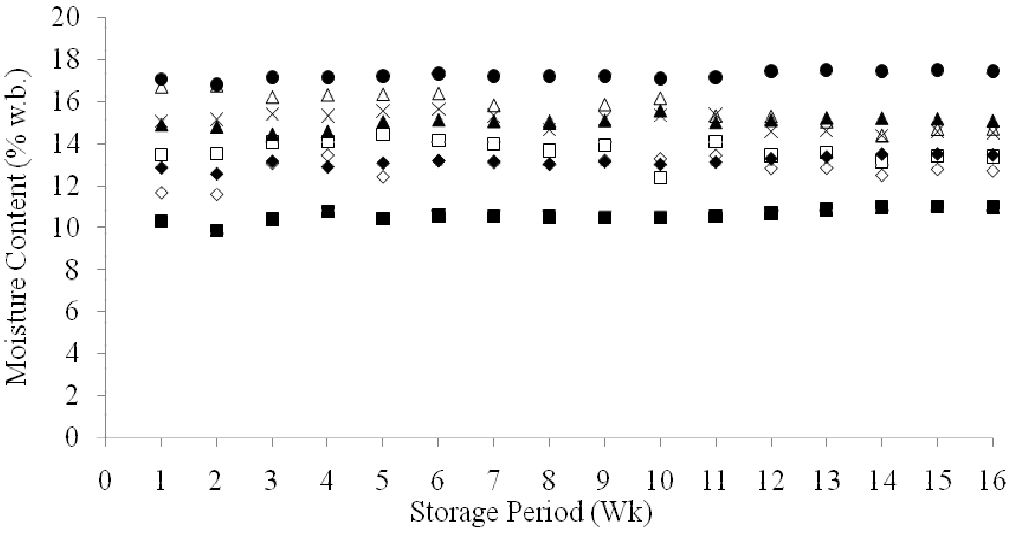


(a) 10°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □12.5%, ×15.0% and △17.5%.



(b) 20°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

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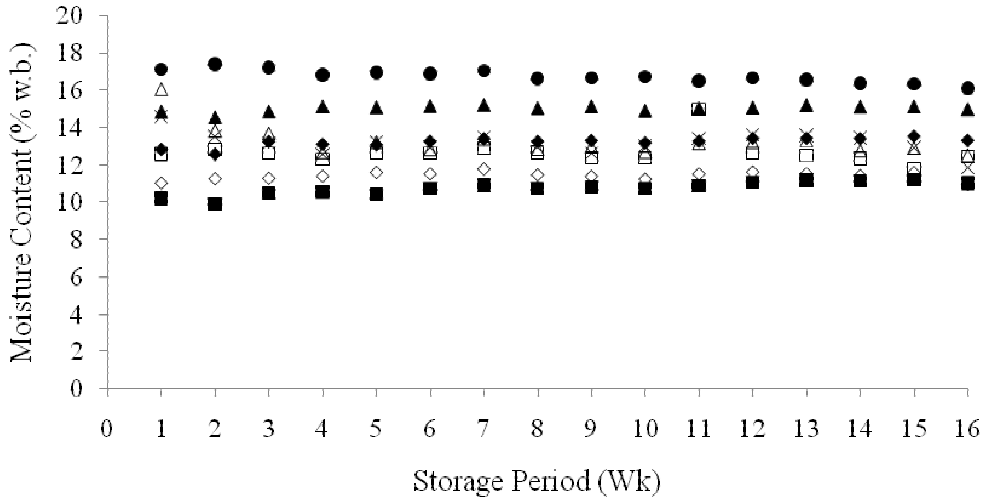
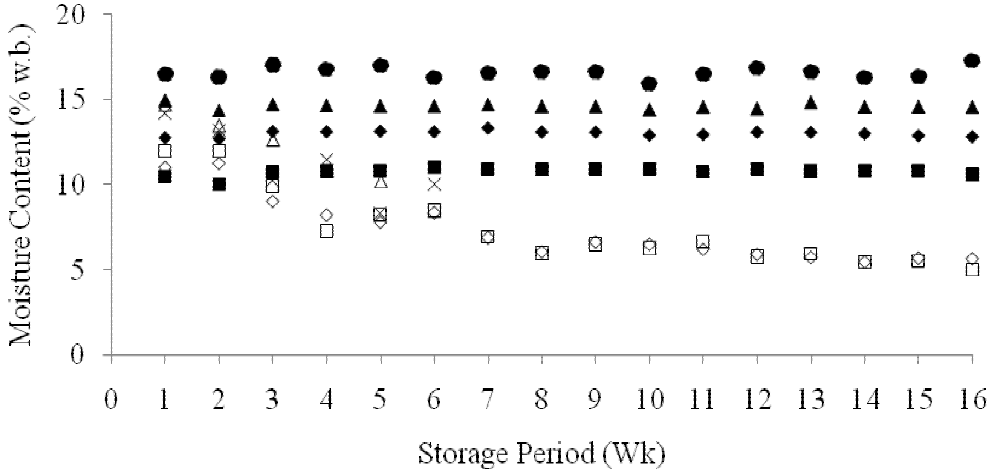


(c) 30°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □ 12.5%, ×15.0% and △17.5%.

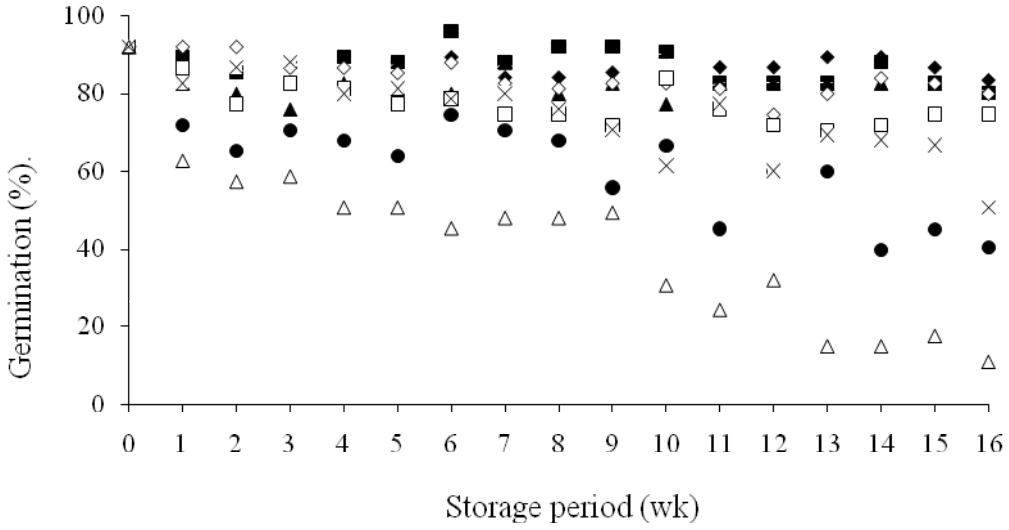


(d) 40°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □ 12.5%, ×15.0% and △17.5%.

Fig. 1. Comparative changes in moisture content (% wb) with respect to storage period at 10°C (b) 20°C (c) 30°C (d) 40°C



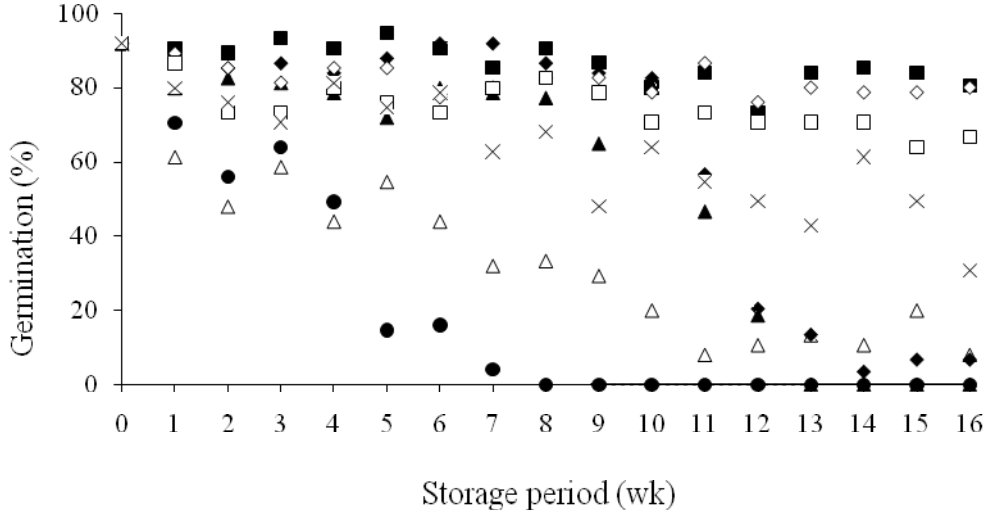
(a) 10°C

Current study (Case 1)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5%

Sathya et al. (2008) (Case 2)

◇10.0%, □12.5%, ×15.0% and △17.5%.



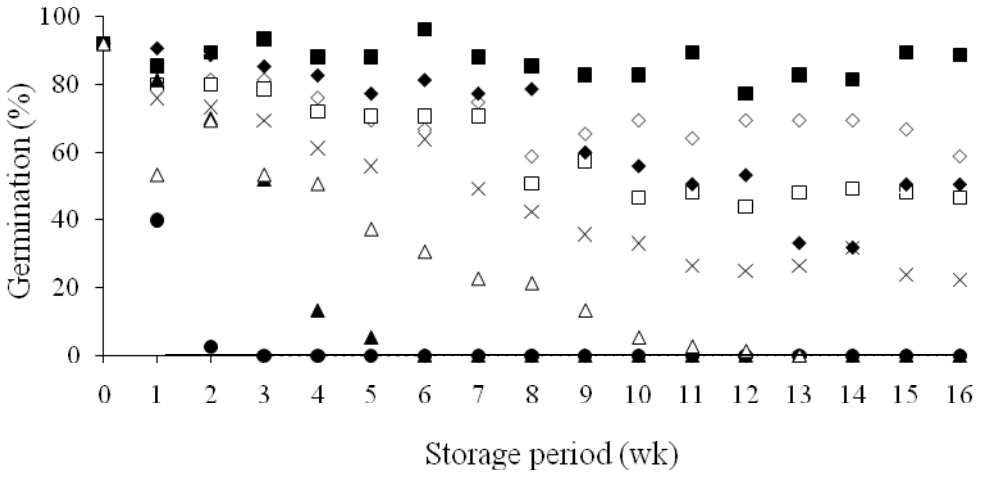
(b) 20°C

Current study (Case 1)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5%

Sathya et al. (2008) (Case 2)

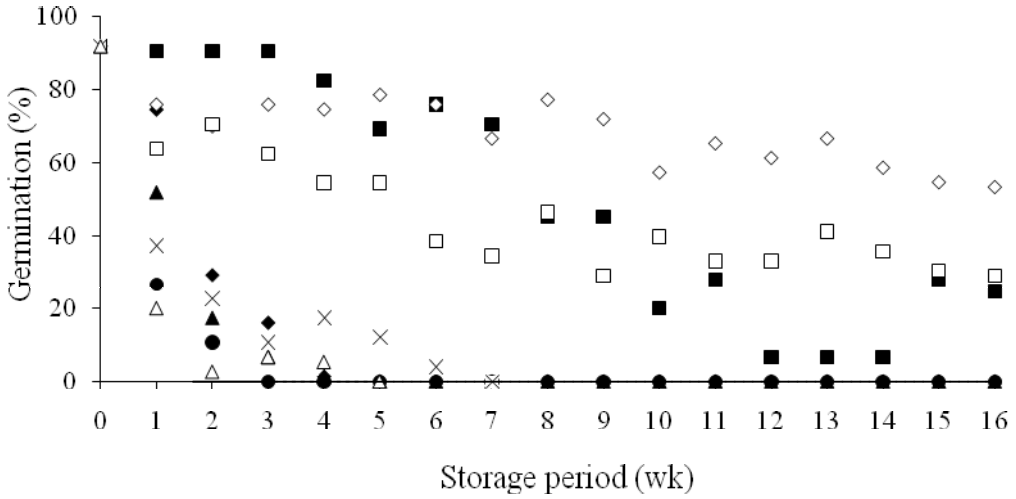
◇10.0%, □12.5%, ×15.0% and △17.5%.



(c) 30°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □12.5%, ×15.0% and △17.5%.

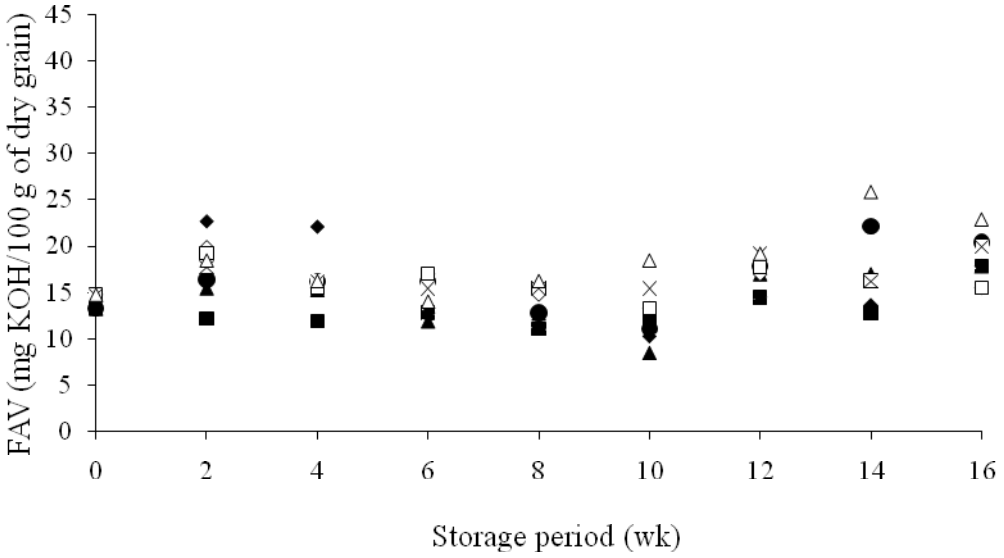


(d) 40°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □12.5%, ×15.0% and △17.5%.

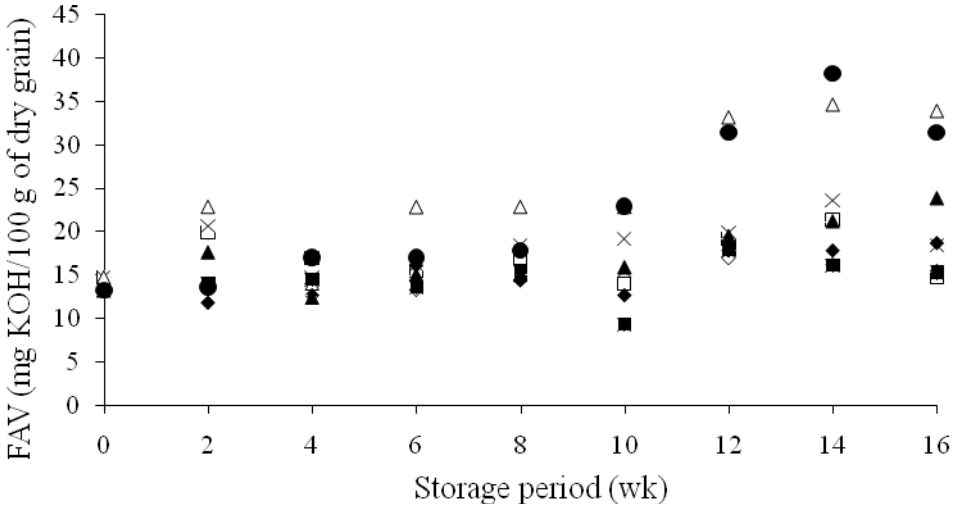
Fig. 2. Comparative changes in germination with respect to storage period at (a) 10°C (b) 20°C (c) 30°C (d) 40°C



(a) 10°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

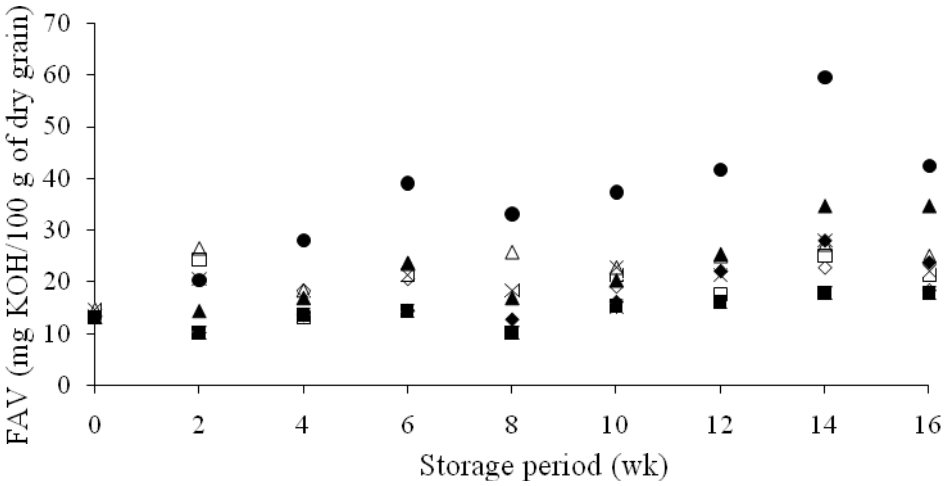
■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □ 12.5%, ×15.0% and △17.5%.



(b) 20°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

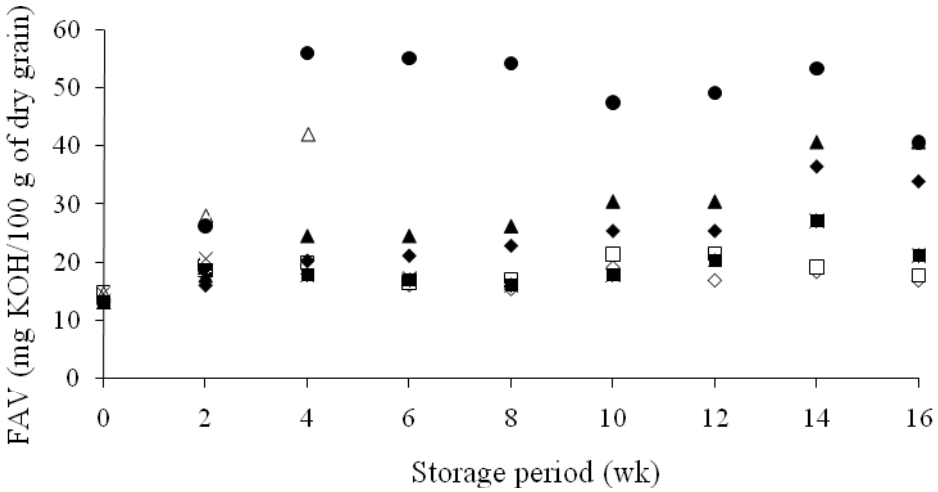
■10.0%, ◆12.5%, ▲15.0% and ● 17.5% ◇10.0%, □ 12.5%, ×15.0% and △17.5%.



(c) 30°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ●17.5% ◇10.0%, □12.5%, ×15.0% and △17.5%.



(d) 40°C

Current study (Case 1) Sathya et al. (2008) (Case 2)

■10.0%, ◆12.5%, ▲15.0% and ●17.5% ◇10.0%, □12.5%, ×15.0% and △17.5%.

Fig. 3. Comparative changes in FAV with respect to storage period at (a) 10°C (b) 20°C (c) 30°C (d) 40°C

Table1. Time of the first appearance of visible mould (wk) and respective germination rate in rye (%)

Initial moisture content (% wb)		10.0		12.5		15.0		17.5	
Temperature (^{o}C)	Replicate	Case ψ		Case ψ		Case ψ		Case ψ	
		1	2	1	2	1	2	1	2
10	a	-	-	-	-	-	-	2, 18*	2, 60
	b	-	-	-	-	-	-	2, 14*	2, 52
	c	-	-	-	-	-	-	2, 17*	2, 60
20	a	-	-	-	-	-	-	1, 76	1, 60
	b	-	-	-	-	-	-	1, 64	1, 56
	c	-	-	-	-	-	-	1, 72	1, 68
30	a	-	-	9, 68	9, 56	4, 3*	5, 56	1, 32	1, 52
	b	-	-	9, 60	9, 64	4, 1*	5, 48	1, 44	1, 52
	c	-	-	9, 52	9, 52	4, 6*	5, 64	1, 44	1, 56
40	a	8,48	10, 64	3, 32*	5, 40	1, 40*	1, 36*	1, 68	1, 16*
	b	8,72	10, 48	3, 8*	5, 52	1, 62*	1, 40*	1, 76	1, 24*
	c	8,16	10, 60	3, 8*	5, 72	1, 54*	1, 36*	1, 72	1, 20*

ψ Case 1 - Current study; Case 2 – Study of Sathya et al. (2008)

*Visible mould might have occurred before this time in these cases because of the length of time interval between sampling dates