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ORIGINAL ARTICLE

Effect of storage method and opening time on protein, starch, zeleny value, and mold level of stored wheat

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ABSTRACT

The effect of hermetic storage methods and opening time on protein, starch, zeleny value, and mold level of stored wheat *is not extensively documented in Ethiopia. Therefore, the present study was conducted to evaluate the effects of hermetic storage methods and their opening time on the performance of stored wheat. The experiment consisted of six storage methods, namely metal silos, Purdue Improved Crop Storage bag, Super GrainPro bag, Plastic drum, Triplex applied to wheat in Plastic drum, and Polypropylene bag as control with three opening times, two, four, and six months, were evaluated. Protein, starch, wet gluten, Zeleny, and mold colony-forming unit were determined after one year of storage. Full factorial design was used, and the ANOVA was determined using R software. Results indicated that storage methods such as PICS, Super GrainPro bags, Triplex applied to drum plastic, and plastic drums led to significantly (P<0.05) lower levels of mold growth compared to the control and the metal silo. The present study showed that opening time had a significant effect on wet gluten and mold growth of wheat grain properties. Thus, the opening time must be considered during the storage of grains.*

Key words: *Hermetic storage, Wheat, Nutritional composition, Opening time, Mold*

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1. Introduction

Cereals, which include wheat, rice, corn, sorghum, millet, barley, rye, and others belong to the monocot *Poaceae* family, also referred to as the Gramineae family. They have long, thin stalks and are rich in fiber, protein, starch, and other micronutrients, particularly zinc and magnesium (Sarwar, 2013). According to Saeid (2017), it is a source of fiber, ash, protein, fat, carbohydrates, and vitamins and minerals. The most extensively produced grain and staple food crop worldwide is wheat (*Triticum aestivum*) (Saeid, 2017). Over 90% of the food produced and almost 70% of the calories consumed by people in developing nations come from wheat (Kumar & Kalita, 2017). Next to maize and teff, wheat is the most widely produced and staple grain in Ethiopia (Dessalegn et al., 2017). Wheat preservation is essential for enhancing food security because it increases the cereals' storability after harvest. Regrettably, insufficient storage methods have led to significant postharvest losses of wheat grains (5-26%) (Dubale, 2019). There is a significant impact on global food security from the 25–33% of grain crops that are lost annually during storage, and the 49% of grain crops that are kept in Ethiopia by conventional storage methods (Alemayehu et al., 2023). Among the major problems with storing wheat grains is insect festination. Insect damage and even destruction of food components can occur when wheat grain is stored improperly after harvest (Martin et al., 2015). Birds, termites, and mold growth are additional factors in addition to insect infestation.Different ancient methods, including the use of polypropylene bags and 'gota' or 'gotera', have been used by Ethiopians to preserve their commodities (Kasahun Olana, 2021). But the grain's storability cannot be increased; instead, weevils and insects cause a very large loss. Alternative approaches, such as synthetic compounds that are hazardous to human health and the environment, have been employed to lessen the loss of wheat grain. As a result, various ecologically safe storage techniques have emerged recently to lessen the aforementioned issues without the use of chemicals (Worku et al., 2019). Various hermetic storage techniques can extend the grain's storability (Chattha et al., 2015, Hocking., 2003). Therefore, the best course of action for keeping the grain could be to look for safe storage. Additionally, Worku et al. (2019) studied the physicochemical characteristics of stored wheat, while Kalsa et al. (2019) studied the effects of hermetic storage techniques on grain deterioration and weight-loss after six months of storage. Nonetheless, limited investigation is carried out on wheat grain storability by assessing the effectiveness of hermetic storage methods after a six-month storage period. Thus, the aim of this work is the evaluation of storage method and opening time on protein, starch, and zeleny value and mold level of stored wheat after six months of storage. This work is important for upcoming researchers, agricultural experts, policymakers, and farmers to preserve their grains by integrating the hermetic storage method with opening time.

2. Materials and Methods

2.1. Raw material collection, preparation and experimental site

Wheat grain of *kakaba* variety was obtained from the Ethiopian Seed Enterprise, Bahir Dar Center, Ethiopia. The wheat grain was cleaned to remove broken kernels, chaffs, dusts, then stored for one year (February 2018 – February 2019) in different storage methods under ambient conditions with (temperature of 24.6±0.6°C and relative humidity of 48.2±10.4%) at the food engineering laboratory in the faculty of chemical and food Engineering, Bahir Dar institute of technology, Bahir Dar University.

2.2. Experimental Design and Treatments

In this investigation, a completely randomized design (CRD) was employed. Metal Silo, Purdue Improved Crop Storage Bag, Super GrainPro Bag, Plastic Drum, Plastic Drum with Triplex powder at a rate of 0.25% (w/w), and Polypropylene Bag, with three level of opening times (two, four and six months) and the experiment was carried out with replication and the total treatment was 54.

2.3. Experimental Analysis

2.3.1. Sampling

One kilogram of wheat sample was taken from each storage methods and the polypropylene (woven) bag containers) at the top, bottom, and middle of the storage at each opening time.

2.3.1.1 Sample treatment

After collection of wheat grain samples from different storage methods, the samples were sieved to do the required wheat grain parameters.

2.4. Data collection

2.4.1 Nutritional composition (Starch and Protein) analysis

The protein and starch content of wheat grain for each stored samples were measured by using Infratec1241 Grain Analyzer (FossTecator, Sweden). The instrument had calibrated for different wheat grain and the protein and carbohydrate content of wheat samples were measured at a time. The percentage of each sample was adjusted to the standard moisture content using equation (1).

$$\text{Adjusted starch } (\%) = \frac{100 - \text{Mc}}{87} \text{ x starch content} - - - - - - - - - - - - - (1)$$

Where: Mc is moisture content of wheat grain

2.4.2. Technological properties (Wet-Gluten and Zeleny)

The percentage of both wet gluten and Zeleny were measured as protein and starch following the same procedures as determination of nutritional composition. The percentage of each sample was adjusted to the standard moisture content using the equation (1) described for in starch content section 2.4.1.

2.4.3. Mould growth

The determination of mold growth for each wheat grain sample was determined according to the method described by (Malomo, Ogunmoyela, & Oluwajoba, 2012). Wheat grain samples were ground using a laboratory grinder. Ten gram of wheat flour sample was taken from each treatment combination. Every sample was then transferred to 90 ml of maximum recovery solution. Sample mixture was serially diluted by taking 1ml volume of sample wheat sample to 9 ml volume of maximum recovery and continued to other test tubes. Sterilized and cooled potato dextrose agar (PDA) mixed with 10% of tartaric acid was used as growing media. The PDA-tartaric acid mix was poured to a Petri dish until it covered the surface and allowed to solidify. 0.1 ml volume of from each serial dilution of wheat flour sample was transferred to the solidified agar and spread using road glass as a spreader. Petri-dishes were kept in an incubator at 25 ℃ for five days, and the numbers of mould colonies were counted using colony counter (Funke Gerber, Germany).

3.5. Data Analysis

The data obtained were subjected to a two-way analysis of variance (ANOVA) using R software version 4.3.3. Mold colonies were log transformed before analysis. Least significant difference (LSD) tests at a 5% level of significance were used to separate the means when there were significant differences at $p<0.05$.

4. Results and Discussion

4.1. Protein

The different storage methods and their opening times did not show a significant difference ($p = 0.16$, $p=0.81$) respectively on the protein content of stored wheat samples(Table1).Even though storage methods and their opening times did not show significant differences, the protein content of wheat grains may vary between 10% to 18% of the total dry matter (Saeid, 2013),another studies indicated the protein content of wheat varies between 10 and 20% (Mutlu, Hakki, & Huseyin, 2011) and therfore,the present results agree with the reported value,the average of "kakaba" wheat grain protein before storage is 13.31±0.2% (Soboka S &Ethicha F, 2017).This could be the reason that, due to storage loss associated with insect and mould contaminations.Many pests may eat the bran of cereals reducing vitamins, rodents and moth larvae attack the germ of the grain thus removing a large percentage of the protein (Shiju, 2010) in this case protein content should be reduced.

Table1.Main effect of storage methods on protein content and starch composition of wheat grain from different hermetic containers and their opening times.

Storage methods	Protein (%)	Starch (%)
super Grain pro bag	11.1± 0.1[a]	66.6±0.9 [a]
Metal silo	11.3± 0.2 [a]	65.5±0.4 [a]
Plastic drum	11.1±0.1 [a]	66.6±0.4 [a]
Plastic drum +triplex treated	11.2±0.2 [a]	66.5±0.2 [a]
Perdue improved crop bag	11.10±0.2 [a]	66.5±0.3 [a]
polypropylene bag	11.2±0.3 [a]	64.6± 0.5 [a]
F (5, 34)	22.75	1.530
P-value	0.16	0.96
Opening time	Protein	Starch
2 months	11.2±0.1 [a]	66.6±0.6 [a]
4 months	11.1±0.2 [a]	66.5±0.3 [a]
6 months	11.2±0.2 [a]	66.5±0.4 [a]
F (2, 34)	0.21	0.89
P-value	0.81	0.24

Means within a column followed by the same letters are not significantly different at 5% level of significance**.**

4.2. Starch

The different storage methods and opening times also did not show a significant difference ($P = 0.96$, $P = 0.24$) respectively on the starch content of stored wheat (Table1). The result is in agree with similar work reported, the amount of starch contained in wheat grains may vary between 60% and 75% of the total dry weight of the grain (Saeid, 2013).However, the loss of wheat grain sample is highest in polypropylene bag followed but it is not much lower than any other hermetic storage methods this due to the insect body part and its metabolic wastes remain inside grain could increase the starch content of wheat grains(Keskin & Ozkaya, 2015).

4.3. Wet gluten

Based on the result there was a significant difference ($p < 0.01$, $p = 0.03$) due to both different storage methods and their opening times on the wet-gluten content of stored wheat (Table2). The average value of wet gluten content of wheat grain is lower than the literature value (33%)(Başlar & Ertugay, 2011). This may be due to mostly the value depends on the type of species wheat that used to conduct the study. According to Soboka .S &Ethicha.F, (2017)the average wet-gluten content of "kakaba'' wheat grain is about 30.29±0.95% at 12.5 % moisture content which is much higher in the present findings. From this, it can be discussed that there was grain loss during one-year storage which may cause the reduction of wet-gluten contents.

Table 2: Main effect of storage methods on wet-gluten and Zeleny value of wheat grain from different hermetic containers and their opening times.

Storage methods	Wet gluten (%)	Zeleny (ml)
Super Grain pro bag	28.6 ± 0.3^{a}	31.0 ± 1.5^{a}
Metal Silo	26.9 ± 1.8^{b}	31.1 ± 0.6^{a}
Plastic drum	28.3 ± 0.8^{a}	31.6 ± 0.4^{a}
Plastic drum +triplex treated	27.9 ± 0.5^{a}	31.3 ± 0.5^{a}
Perdue Improved Crop bag	28.5 ± 0.6^{a}	31.8 ± 0.3^{a}
poly propylene bag	28.0 ± 1.00^{a}	31.7 ± 0.9^{a}
F(5,34)	3.58	1.21
p-value	0.01	0.32
LSD	1.50	
Opening month		
2 months	27.9 ± 1.0^{a}	31.5 ± 0.5^{a}
4 months	28.5 ± 0.4^{b}	31.5 ± 0.8^{a}
6 months	27.8 ± 1.3^{a}	31.4 ± 1.1^{a}
F(2,34)	3.26	0.02
P-value	0.03	0.97
LSD	1.54	

Means within a column followed by the same letters are not significantly different at 5% level of significance.

4.4. Zeleny test (Sedimentation value)

Based on the analyzed result there were no significant differences in different storage methods and their opening times on Zeleny content of stored wheat samples ($P = 0.32$, $P = 0.97$) respectively (Table 2). From the present results, Zeleny test or sedimentation value found in the range of 30.1-31.8 ml which is much lower than the reported "kakaba"

sedimentation value of wheat grains and in agreement of findings, the average sedimentation value of kakaba wheat grain is 72.74±1.23 (Soboka S & Ethicha F, (2017).This indicates that the stored grains may not be used for bread production due to a low value of sedimentation values and wet-gluten contents. Higher settlement in a slightly acidic medium is important for leaved products which also resulted from different composition and granulation of tested components (Hrušková et al., 2002). The sedimentation value may affect by the type of storage methods and how long it stores or duration of storage. Since the present samples were stored one year fully which may a reason for the reduction of sedimentation values due to protein degradation of stored wheat by insects.
Protein and sedimentation value (Zeleny) have a direct relation. A similar study reported that both have a correlation between protein and Zeleny (sedimentation value) (Famra, 2003). The sedimentation index (Zeleny test), which measures the sedimentation volume of gluten in the flour dispersion is a function of its gluten content and the gluten quality (Mutlu et al., 2011).

4.5. Mould Growth

There were significant differences ($p = 0.01$) due to storage methods on mould growth of stored wheat grain. The maximum mould colony was found in polypropylene bag significantly and the lowest was in a triplex applied to plastic drum (Table 3). From this, it can be discussed that hermetic storage methods are important to control not only insects but also it is important to control the growth of moulds. Hermetic storage methods used to keep grains with a less mould deterioration (Somavat, 2016). When the storage is safe the contamination and production of toxic components of wheat grain can be reduced. Similar works reported that good postharvest practice can reduce the health risk due to mould growth and potential toxin contamination (Sadhasivam et al., 2017).

The moisture content and water activity of wheat samples are the main factors for mould growth of wheat grain during storage Similar works reported that mould activity will, in turn, raise temperatures and result in an increased rate of insect reproduction and greater numbers of insects create more moisture and finally insects also cause quality deterioration through their excreta as they consume (Befikadu,2014)..

In polypropylene bag, the number of mould was highest due to maybe insect contamination which favors the growth of mould. Somavat et al., (2017) conducted that, insect contamination favors the growth of microorganisms especially moulds. Moreover, moulds cause for rapid deterioration in the quality of wheat grains due to is improper storage methods. Reduction of the nutritional value of stored grain, possibly accompanied by undesirable fungal contamination and, consequently, toxin production (Szumiło et al., 2010). Therefore, the present result shows that polypropylene bag is the potential mould contamination which will lead to the formation of toxic components which are unsafe for consumption. Thus, the results shows agree with the report made by Kiaya, (2014), toxic substances elaborated by molds (known as mycotoxins) cause a loss in food quality and nutritional value.

Opening times had a significant difference ($p = 0.01$) on the mold colony of stored wheat grains. The highest and lowest value of mould colony was observed at two and six months (Table3). Here opening times also affect the mould growth of stored wheat as storage methods. This may be related to when storage methods open earlier the growth of insects will increase which allows the formation of mould contamination due to the access of oxygen.

Table 3 Main effect of storage methods on mold colonies of wheat grain from different hermetic containers and their opening times.

Storage methods	Number of mold (CFU)
Super grain pro bag	3.6±3.8[b]
Metal bin	5.9± 5.4[ab]
Plastic drum	4.3± 4.2[ab]
Triplex applied to a plastic drum	2.9±2.9[b]
Perdue improved crop bag	3.9± 4.0[ab]
Poly propylene bag	11.9±11.5[a]
F(5,34)	1.82
p-value	0.01
LSD	0.36
Opening time	Number mold (CFU)
2 months	9.4±8.7[a]
4 months	3.7±3.4[b]
6 months	2.7±3.1[b]
F(2,34)	6.66
P-value	0.01
LSD	0.36

Means within a column followed by the same letters are not significantly different at 5% level of significant difference.

5. Conclusion

In this study storage and opening time was not show any interaction effect on Protein, starch and zeleny value and mold level of stored wheat grain and hence only the main effect was discussed in this work. When compared to the other storage methods, the polypropylene bag utilized as the control in this study demonstrated the lowest amount of starch percentage and the highest number of mould (CFU)in the stored wheat. Within the hermetic storage methods, the highest mould level was observed in metal silo than other hermetic storage methods, due to air leaks at the start of storage. Therefore, the metal silo used in hermetic storage methods was ineffective in preserving the wheat grain. When the airtightness is verified, smallholder farmers are strongly encouraged to use metal silos since they are mechanically stronger than any other hermetic storage technique. Applying botanical plant leave powder, such as triplex powder, to a plastic drum can improve wheat grain storage, much like other hermetic storage techniques. Furthermore, from the work, opening times did show significant effect on wet gluten, and mould level of wheat grain even though the influence of opening time on the performance of storage methods is not well studied therefore, it is better to take it under consideration during storage of grains. Finally extra research work is required whether opening time has an effect or not on the starch, protein zeleny value and mould development of grain stored with hermetic storage methods.

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