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ORIGINAL ARTICLE

Evaluation of Proximate composition, Microbial load and colour of Green gram during storage under flexible hermetic and Conventional packaging materials

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ABSTRACT
Maintaining the seed quality is the major challenges during long-term storage of green gram. Flexible hermetic bags are effective in maintain the seed quality. The objective of this study was to evaluate the quality of green gram stored in hermetic and non-hermetic storage system. Hermetic SuperGrain bag (SGB), and conventional bags such as low density polyethylene (LDPE), woven polypropylene (WPP) and jute gunny (JG) bags were filled with 5 kg of green gram sample with 13.29% moisture content. SGBs and LDPE bags were hermetically sealed while WPP and JG bags were tied by sisal twine. The bags were stored at room environment (temperature: 26 ± 2ºC; RH: 50 ± 5%) for 6 months. Moisture content, colour changes, proximate composition and microbial load were evaluated during storage. The results showed no significant changes in moisture content under SGBs (13.20%) whereas it reduced in LDPE (12.46%), WPP (9.21%) and JG bags (9.16%). Protein content in SGBs, LDPE, WPP and JG bags were 23.25, 21.53, 19.77 and 18.79% respectively. Fat (1.29%) and ash content (3.36%) did not differ significantly during storage in all the bags. Carbohydrate level was significantly lower in SGBs (58.90%) than in LDPE (61.38%), WPP (66.38%) and JG bags (67.40%). No significant changes in surface colour (ΔE) under SGBs (42.92) and LDPE (42.75) whereas WPP (42.24%) and JG bags (42.17) showed significant reduction. Bacterial and fungal counts were extremely low in SGB as compared to LDPE, WPP and JG bags.

Keywords: *Green gram, SuperGrain bags, WPP bags, Proximate components, Colour, Microbial load*

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INTRODUCTION

Green gram, *Vigna radiata* (L.) R. Wilczek (Fabaceae), also known as mung bean, is one of the important legume crops in tropical and subtropical regions. India is the world's largest producer of green gram and produces around 1.5 to 2.0 million tons annually which is 70% of the world's total production [1]. Green gram is an excellent source of protein, which is almost three times that of cereals. It is also a good source of carbohydrate, fat, fibre, minerals and vitamins [2]. It is consumed in the form of whole beans, dehusked split dal, powdered form, as well as number of recipients, which is an essential supplement of cereal based diet. Green gram is a seasonal crop, which produced during kharif season in North India and *rabi* season in South India. There is a sizeable quantitative and qualitative loss during storage due to improper and inefficient methods of storage. The proper storage method secures supply of green gram round the year by reducing losses. Several indigenous storage options such as metal bins, earthen bins, bamboo baskets pasted with mud, drums, polyethylene bags and jute gunny bags are being used [3]. Moisture content is one of the important factors that influence in the quality during storage and also enhances microbial activity, which in turn contribute to heating up the grain [4]. Seed is a hygroscopic entity. Water present in the seed vaporize when the ambient air temperature increase and relative humidity decrease thus the seeds will lose moisture. Alternatively, if the water vapour pressure in the grain is less than the atmospheric water vapour pressure, grain absorbs moisture from atmosphere. This hygroscopic nature leads deteriorate the quality [5].

Type of packaging materials also have a significant effect on the grain moisture content. The characteristics of packaging materials such as water vapour transmission rate (WVTR) and oxygen transmission rate (OTR) are related to moisture content, quality and microbial load of the commodity. Different packaging materials have different WVTR and OTR. Woven polypropylene and jute gunny bags have large pore size that allows free diffusion of oxygen and water vapour result changes in grain moisture content and quality within them [6]. The low density polyethylene (LDPE) bags have WVTR of 16-23 g/m^2/day at 38ºC and 90% RH and OTR of 7000-8500 cc/m^2/day at 23ºC and 0% RH [7]. Hermetic SuperGrain Bag has very low OTR (≤ 4 cc/m^2/day at 0.1 MPa) and WVTR (≤ 5 g/m^2/day) and can be used to store food grains safely [8]. The SuperGrain bags are excellent O_2 barrier and reduced storage losses of cowpea in Niger as compared to woven plastic bags [9]. Maize stored in SuperGrain bag retained onset moisture content (12%), grain quality and no fungal growth [10]. Hermetic SuperGrain bag maintained the quality and onset moisture content of wheat (9%), maize (9.2%), quinoa (7.5%) and cotton seed 7.65%) up to 70% RH however the moisture content increased to some extent (1-2%) at higher level of RH [6].

As conventional packaging materials are porous in nature, dried grains in these materials can regain moisture under high ambient relative humidity. SuperGrain bags are made up of multi-layer high strength polyethylene with very low water vapour and oxygen transmission rate. Potential of a flexible packaging material in long-term storage of grains may be quantified based on its ability to preserve the quality of products. In this study, proximate composition, microbial load and colour of green gram during storage under different flexible packaging materials such as SuperGrain bags, low density polyethylene bags, woven polypropylene and jute gunny bags were evaluated.

MATERIALS AND METHODS

Green Gram

The study was conducted at the Department of Farm Structures, College of Agricultural Engineering and Technology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. Recently harvested green gram of the cultivar NVL-1 was procured from a local farmer cum dealer at Pahadpur village, Akola. The whole green gram was cleaned manually, graded using sieve and foreign matters were removed. The green gram was naturally sun dried to 13% moisture content and used in the experiment. The experiments were conducted during August 2016 to January 2017 under room temperature of 26 ± 2ºC and relative humidity of 50 ± 5%.

Packaging Materials

SuperGrain bags (SGBs), low density polyethylene (LDPE) bags, woven polypropylene (WPP) bags and jute gunny (JG) bags were tested in this study. GrainPro SuperGrain packaging combines an inner bag with an outer protective bag. The inner bag is a multi-layer polyethylene with a thickness of 78 μm and outer bag is a standard woven polypropylene or jute bags [9]. The characteristics of SGB and LDPE packaging films such as WVTRs and OTRs are presented in the introduction section. SGB bags were procured from the Pest Control India Private Limited, Bangalore, India whereas LDPE, WPP and JG bags of same capacity purchased from a local trader at Akola.

Experimental Procedure

The storage bags were carefully inspected for stitching defects and sealing blemishes in order to ensure that good quality bags were used. The treatment combinations were: T_1 - SGBs; T_2 - LDPE bags; T_3 - WPP bags; and T_4 - JG bags. The treatments were arranged in a completely randomized design with three replications. All the storage bags were filled with 5 kg of the green gram. The excess air in the SGB was removed (according to manufacturer's instructions) by pressing down the plastic to the beans. The SGB and LDPE bags were sealed hermetically with an automatic multi-purpose sealing machine (Model AMS-100A, Print Packaging Systems, Mumbai, India). The WPP and JG bags were closed by twisting the loose end and tying tightly with sisal twine. The bags were arranged in treatment-wise and kept in the laboratory at room environment (temperature: 26 ± 2ºC, RH: 50 ± 5%) for 6 months.

Moisture Content Determination

Moisture content of the green gram was determined using digital moisture testing machine (Model: 6005/40472, Osaw Industrial Products Pvt. Ltd., Haryana, India). About 30 g representative green gram sample was taken in volume cup-A, and transferred it into the test cup and placed it in the housing of the compression unit. The sample in the test cup was compressed to a specified thickness with the help of a Ratchet handle. The thickness can be read on the linear vertical scale and circular scale provided in the machine. The moisture content was recorded in % (dry basis) through display unit by pressing the press button.

Surface Colour Analysis

Colour is often used as an indication of quality of freshness for food product. Hence, surface colour of the green gram was analysed before and after the storage to check any changes in it during storage under selected packaging materials. The surface colour was measured using Ultra Scan VIS Hunter Lab (Hunter Associates Laboratory Inc., Reston VA., USA). A glass cell containing green gram samples was placed against the light source, covered with a black cover and 'L', 'a', and 'b' colour values were recorded. The L* is the lightness coefficient, ranging from 0 (black) to 100 (white) on a vertical axis. The a* is purple-red (positive) and blue-green (negative) on a horizontal axis. A second horizontal axis is b* represents yellow (positive) or blue (negative). Total colour change (ΔE) was calculated using following formula as defined by Vunnam et al [11].

$$\Delta E = \sqrt{\left(L_f^* - L_s^*\right)^2 + \left(a_f^* - a_s^*\right)^2 + \left(b_f^* - b_s^*\right)^2}$$

where, ΔE is the total change in colour, L_f is the brightness value of sample before storage, L_s is the brightness value of sample after storage, a_f is the hue value of sample before storage, a_s is the hue value of sample after storage, b_f is the chroma value of sample before storage and b_s is the chroma value of sample after storage.

Proximate Composition Analysis

Proximate composition such as protein, fat, ash, carbohydrate and moisture content were analysed before and after storage to check any changes in quality of green gram during storage. The protein content was determined by micro-Kjeldahl method as described in AOAC [12]. The fat content was estimated using the standard extraction method AOAC [12] using petroleum ether as the extraction solvent employing SOCS plus extractor (Pelican, India). The ash content was estimated by using standard method of AOAC [12].

% Carbohydrate = 100% - (moisture + protein + fat + ash)

Microbial Load Analysis

Microbial analysis of green gram samples was done for fungal and bacterial load after six months storage to check the microbial activities. The samples were analysed for bacterial and fungal load by Standard Plate Agar [13] and dilution plating on Martin Rose Bengal Agar [14] respectively.

Statistical Analysis

A completely randomized design was used with three replications. The data for different treatment combinations and storage times were subjected to analysis of variance using SAS software. To test the difference between the average values, least significant difference method was adopted and the significance was defined at the level of 5%.

RESULTS AND DISCUSSION

Moisture Content

Average moisture content of green gram was 13.29% at the onset storage (Table 1). After 6 months, there was no significant change in moisture content of green gram stored in SGBs (13.20%). On the other hand, the moisture content of green gram stored in LDPE, WPP and JG bags were 12.46, 9.21 and 9.16% respectively (Table 2). The result showed SGBs maintained the onset moisture during storage whereas reduced significantly ($P \leq 0.05$) in LDPE, WPP and JG bags. Moisture content is one of the main factors that encourages grain deterioration, growth of insects and fungi during long term storage. Hence, safe storage moisture content should be maintained to protect their nutritional quality. As the SGB has very low WVTR (≤ 5 $g/m^2/day$), diffusion of moisture between inside bag and outside environment may be greatly prevented. Whereas LDPE bags may be allowed moisture to diffuse due to its high WVTR (16-23 $g/m^2/day$). Therefore moisture content of green gram in LDPE bags slightly reduced to 12.46%. On the other hand, WPP and JG bags showed a great decrease in moisture content over the same period. The water present in the beans vaporizes in response to ambient temperature and RH (< 55%) subsequently lost moisture. Similar results were reported by Likhayo et al [10] that the moisture content of maize stored in SuperGrain IV-R™ bags remains unchanged during 180 days of storage but the moisture content of maize decreased over the storage period in the polypropylene bags.

Surface Colour

The SGBs and PE bags had no significant changes in surface colour of the green gram, whereas WPP and JG bags showed a significant reduction in colour change as compared to initial level. The initial surface colour value of green gram (ΔE) was 43.00. The changes in surface colour (ΔE) of the stored green gram were 42.92, 42.75, 42.24 and 42.17 in SGBs, LDPE, WPP and JG bags respectively after 6 months of storage (Fig. 1). Colour is often used as an indication of quality of freshness for stored products. In our study, the ΔE value of green gram stored in SGBs were on far with initial ΔE value indicates that maintained freshness whereas the ΔE values of green gram stored in WPP and JG bags were significantly reduced. The degradation of colour could be due to oxidation reaction of the anthocyanin and carotenoids. The ΔE value in SGBs showed almost nearby initial ΔE value. As the SGB has very low WVTR (≤ 5 $g/m^2/day$), moisture exchange between inside bag and outside environment may be restricted. In addition, oxygen level inside the bag probably decrease due to its very low oxygen transmission rate (≤ 4 $cc/m^2/day$) thus there may be controlled respiration rate of green gram. Therefore no significant changes in colour of the sample in the SGBs. On the other hand, the result showed a significant reduction in ΔE value of green gram stored in LDPE, WPP and JG bags. Since WPP and JG bags have large pore size, there may be free moisture and oxygen movement between storage and outside environment that leads in degradation of surface colour. Similar findings reported by Walker et al [15] that the maize grain with less than 13.5% moisture content stored under hermetic conditions reduced discoloration as compared to stored in PP bags.

Protein Content

Protein content of green gram before storage was 23.65% (Table 1) and after 6 months of storage in SGBs, LDPE, WPP and JG bags were 23.25%, 21.53%, 19.77% and 18.79% respectively (Table 2). There was no significant changes in protein content of the green gram stored in SGBs (23.25%) as compared to initial level (23.65%) whereas LDPE, WPP and JG bags had significant effect on protein content (SED = 0.048; CD = 0.104; $p \leq 0.05$). The properties of packaging materials such as water vapour transmission and gas transmission rate may have influence in the nutritional quality of stored products. Butt et al [16] reported a decrease in protein content of wheat grain with higher moisture during storage in PP bags for 60 days. Pessu et al [17] reported that the protein percentage of soybean was significantly decreased during long term storage.

Fat Content

The fat content of the green gram before storage was 1.31% (Table 1) and after 6 months storage in SGBs, LDPE, WPP and JG bags were 1.29%, 1.28%, 1.27% and 1.27% respectively (Table 2). The result showed no much significant reduction in fat content during storage (SED = 0.028; CD = 0.054; $P < 0.05$). Samuels and Modgil [18] and Butt et al [16] reported similar finding on fat content of wheat stored in jute and polyethylene bags. Gopinath et al [19] reported that the reduction in fat content of green gram and red gram during storage in cotton bags.

Ash Content

Initial ash content of green gram was 3.41% (Table 1) and after 6 months storage under SGBs, LDPE, WPP and JG bags were 3.36%, 3.35%, 3.37% and 3.38% respectively (Table 2). The results showed a small reduction in ash content during storage as compared to initial however statistical analysis showed the storage bags had no significant effect on ash content. Our result was confirmed with results of Ahmad and Pathak [20] for soybean, Patil and Khan [21] for brown rice and Sethi et al [22] for pigeon pea during long term storage.

Carbohydrate Content

Carbohydrate content of the green gram stored in SGBs, LDPE, WPP and JG bags were 58.90%, 61.38%, 66.38% and 67.40% respectively after 6 months of storage (Table 2) whereas 58.34% before storage (Table 1). The results showed that all the storage bags had significant effect on carbohydrate of stored green gram after 180 days of storage period (SED = 0.337; CD = 0.724; $p \leq 0.05$). Obviously, the level of carbohydrate of green gram was significantly lower in SGBs as compared to LDPE, WPP and JG bags. This is because other proximate components (moisture, protein, fat and ash content) were maintained constantly throughout the storage period in SGBs. For calculation of carbohydrate content, sum of moisture, protein, fat and ash content was subtracted from hundred.

Microbial Load

The bacterial counts were higher in WPP (7.65×10^4) and JG bags (7.90×10^4) as compared to SGBs (1.06×10^4) and LDPE bags (3.54×10^4) after 6 months storage period. The surface fungal counts also high in WPP (3.62×10^2) and JG bags (4.67×10^2) as compared to SGBs (0.34×10^2) and LDPE bags (1.42×10^2)

after 6 months storage period (Table 3). The storage bags had significant effect on bacterial and fungal growth (SED = 0.13; CD = 0.279; p ≤ 0.05). Moreover, among the storage bags, these counts were extremely less in SGBs. The bacterial count of 5-6 fold and fungal count of 3-4 fold observed in LDPE, WPP and JG bags as compared to SGBs. The atmospheric condition inside the packaging materials perhaps a reason for bacterial and fungal growth. SGBs had more efficient to protect the stored green gram against bacteria and fungi attacks as compared to other bags. Ng'ang'a et al [23] observed similar trends in mould growth in PP and jute bags that increased up to six-fold than that in PICS bags at the end of 35 weeks storage.

Table 1: Proximate components of the green gram before storage

Proximate components	Value
Moisture content (%)	13.29 ± 0.01
Protein (%)	23.65 ± 0.08
Fat (%)	1.31 ± 0.03
Ash (%)	3.41 ± 0.08
Carbohydrate (%)	58.34 ± 0.41

Each observation is the average of three replicates

Table 2: Proximate composition of green gram stored in different bags after 6 months of storage period

Treatments	Proximate components				
	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
SuperGrain bags	13.20 ± 0.09	23.25 ± 0.16	1.29 ± 0.05	3.36 ± 0.08	58.90 ± 0.20
200 gauge polyethylene bags	12.46 ± 0.02	21.53 ± 0.53	1.28 ± 0.19	3.35 ± 0.22	61.38 ± 0.16
Woven polypropylene bags	9.21 ± 0.16	19.77 ± 0.46	1.27 ± 0.13	3.37 ± 0.16	66.38 ± 0.77
Jute gunny bags	9.16 ± 0.08	18.79 ± 0.91	1.27 ± 0.13	3.38 ± 0.04	67.40 ± 1.03
P ≤ 0.05	**	**	NS	NS	**
SED	0.021	0.048	0.028	0.019	0.337
CD	0.043	0.104	0.054	0.048	0.724

Mean ± SD, (n = 3), NS = Non significant, ** significantly difference at (P ≤ 0.05)

Table 3: Microbial load in green gram stored in different bags after 6 months of storage period

Treatments	Bacterial count (cfu/g) × 10^4	Surface fungi (cfu/g) × 10^2
SuperGrain bags	1.06 ± 0.06	0.34 ± 0.07
200 gauge polyethylene bags	3.54 ± 0.09	1.42 ± 0.14
Woven polypropylene bags	7.65 ± 0.17	3.62 ± 0.15
Jute gunny bags	7.90 ± 0.15	4.67 ± 0.19
P ≤ 0.05	**	**
SED	0.130	0.1197
CD	0.279	0.257

Mean ± SD, (n = 3), NS = Non significant, ** significantly difference at (P ≤ 0.05)

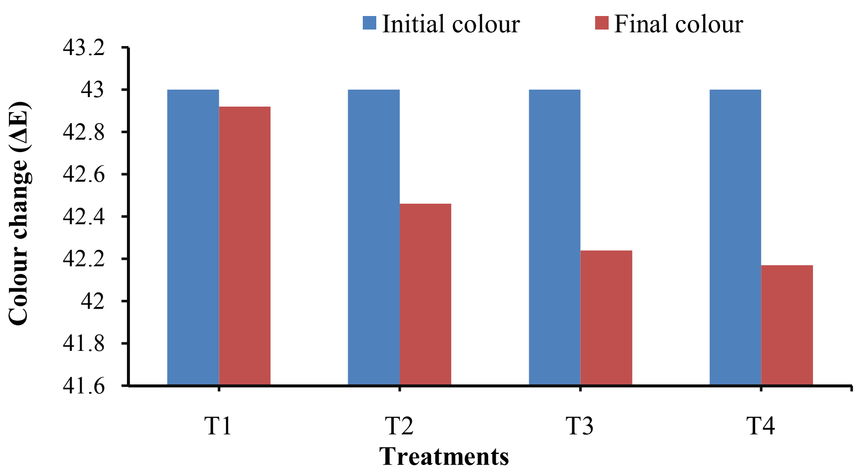

Fig. 1 Changes in surface colour (ΔE) of green gram stored in different bags after 6 months of storage period

CONCLUSION

From this investigation, it is concluded that no significant changes in the proximate components (moisture, protein, fat, ash and carbohydrate content) of green gram stored in SGBs during 6 month period at 26 ± 2ºC and 50 ± 5% RH. Moreover, there was no change in surface colour of the green gram however limited growth of microbial load was observed in SGBs. On the other hand, significant decreasing trend was observed in proximate components and surface colour of the green gram stored in LDPE, WPP and JG bags during the same storage conditions. Besides, these bags showed higher bacterial and fungal counts. Therefore, SuperGrain bags are considered as an effective tool for preservation of green gram.

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