



Evaluation of Elevated CO₂ on Nutritional Quality of Green Gram for the Management of *Callosobruchus chinensis* (L.) during Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author BR conceptualized the study, performed the methodology, did data curation and prepared original draft. Author AG reviewed and edited the manuscript. Authors KSIL, BVJ and CNR wrote, reviewed and did statistical analysis. Author CM edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

An experiment was conducted to study the assessment of nutritional quality parameters of green gram treated with elevated levels of carbon dioxide to manage *Callosobruchus* infestation during 2020-21 at Seed Entomology Laboratory, Seed Research and Technology Centre, College of Agriculture, Rajendranagar, Hyderabad, India. Nutritional quality of *Callosobruchus chinensis* infested green gram seeds exposed to four different concentrations of CO₂ viz., 20, 40, 60 and 80 per cent was studied. The results revealed that the 80 per cent CO₂ concentration maintained high carbohydrate content (62.93 per cent) and crude fat (4.51 per cent) besides less protein (25.26 per cent) content after two months of storage. The 80per cent CO₂ treatment was consistently found to be the best treatment when it was retested in bulk quantity (25 kg of seed) after two months of storage period. High carbohydrate, crude fat content and low protein content were observed in all the CO₂ treatments (20, 40, 60 and 80 per cent) when compared to untreated control. These findings suggest that using elevated CO₂ levels could be a viable alternative to synthetic pesticides chemical fumigants for preserving green gram during storage.

Keywords: Nutritional; *Callosobruchus chinensis*; CO₂; Carbohydrate; protein; crude fat; controlled atmosphere.

1. INTRODUCTION

Legumes, such as chickpeas, play a crucial role in human nutrition, not only supplementing the nutrients in cereal-based diets but also enhancing the taste and texture of staple dishes. Among legumes, green gram, *Vigna radiata* is a crucial pulse crop in India that significantly contributes to both nutrition and the agricultural economy. With its impressive protein content of about 25 per cent, nearly triple that of cereals, this versatile legume serves as a vital source of plant-based protein while also providing substantial economic benefits to farmers across the country. In India, green gram is cultivated over an area of 5.13 million hectares, producing approximately 3.9 million tonnes, which translates to a productivity of 601 kilograms per hectare for the 2020-21 period [1]. Green gram is prone to significant qualitative and quantitative damage caused by storage pests. It is frequently infested by the multivoltine bruchid species *C. chinensis* and *C. maculatus*, which are commonly known as pulse beetles. According to Bhaduria and Jakhmola [2], green gram suffers the highest average damage at 55.4per cent, followed by black gram (35.3per cent), pigeon pea (22.1per cent), cowpea (16.8per cent), gram (11.1per cent) and pea (8.8per cent). However, storing grain for long periods, usually about a year, requires multiple fumigations which can cause chemical residues to build up in the grain [3], leading to the development of resistant populations and increasing costs for farmers. It is crucial to develop natural, affordable and environmentally sustainable methods for managing insect pests in grain and stored

products. In response to these concerns, carbon dioxide has emerged as a promising alternative and it is an environmentally friendly and safe option for grain protection [4] addressing the need for effective pest control methods. Controlled Atmospheres (CA) stands out as one of the most effective preservation techniques for a broad range of agricultural and food products [5]. This approach involves maintaining low oxygen and high carbon dioxide (CO₂) levels, which impact the respiration rates of pests and help prolong the shelf life of fresh produce [6]. The current study was aimed to examine the nutritional quality parameters of green gram treated with elevated levels of carbon dioxide viz., 20, 40, 60 and 80 per cent which involves evaluating various aspects such as carbohydrate composition, protein content and crude fat. Therefore, considering the urgent need to overcome food security challenges, mitigate storage losses due to pests and develop environmentally sustainable pest management strategies, we framed our study to investigate the utilization of carbon dioxide as an effective and eco-friendly seed protectant against the pulse beetle, *C. chinensis*, in stored green gram.

2. MATERIALS AND METHODS

2.1 Mass Culturing of the Test Insect

The mass culturing of *Callosobruchus chinensis* (L.) (Bruchidae: Coleoptera) was conducted in the Seed Entomology Laboratory, Seed Research and Technology Centre (SRTC) at College of Agriculture, Rajendranagar, Professor Jayashankar Telanagana State Agricultural

University, Hyderabad. To establish the stock culture, healthy green gram seeds of the MGG-295 variety were sourced from the Telangana State Seed Development Corporation (TSSDC), Nizamabad, Telangana. The mother culture of *C. chinensis* was maintained at the Seed Entomology Laboratory at SRTC, Rajendranagar, Hyderabad. For mass rearing, approximately 100 adult beetles were introduced into plastic containers holding 1,000 grams of disinfested green gram seeds using an aspirator. The containers were covered with muslin cloth secured with rubber bands to ensure proper aeration and prevent the beetles from escaping. Twenty-five such containers were used for mass culturing and they were kept under laboratory conditions at a temperature of 32 ± 1 °C and relative humidity of 75per cent.

2.2 Experimental Setup

To study the effect of elevated levels of CO₂ on nutritional quality parameters, fifteen airtight containers were filled with five kg of disinfested green gram seed. Twenty-five pairs of freshly emerged adults were released into the containers at twenty-five days prior to carbon dioxide treatment to ensure uniform level of infestation. After twenty-five days, weight of the green gram seed was taken thereafter, CO₂ was released at different concentrations viz., 20, 40, 60 and 80 per cent and three replications of each treatment were maintained. After releasing the CO₂ concentration into the containers, they were made airtight. Untreated control was maintained by the same procedure adopted without exposing the seed to CO₂ concentration. Airtight containers having the disinfested green gram seed exposed to different concentrations of CO₂ were observed in each replication of the treatment.

2.3 Nutritional Quality Analysis

The data on nutritional quality parameters viz., carbohydrate, total proteins and crude fat were observed at two months after treatment. The nutritional quality parameters of the best treatment and untreated control were retested in bulk quantity (25 kg seed) after two months of treatment to ascertain the results.

2.3.1 Carbohydrate of green gram during storage

Estimation of carbohydrates in this investigation was done by the IS 1656-2007 (Difference) method.

Carbohydrates (per cent) = $100 - (A + B + C + D)$

Where,

A = per cent moisture

B = per cent protein

C = per cent fat

D = per cent ash

2.3.2 Total protein of green gram

The protein content of green gram during storage was determined by estimating nitrogen in the sample by micro-kjeldahl method as described by Sadasivam and Manickam [7] using Kelplus auto analyser. Initially in the presence of two grams of catalyst mixture (copper sulphate and potassium sulphate in the ratio of 1: 5) and ten ml of concentrated sulphuric acid (H₂SO₄), 0.2 g of sample was digested at 420°C for two hours. Digested sample was distilled using 40 per cent NaOH in micro-kjeldahl distillation system. Ammonia was observed in excess of four per cent boric acid. The obtained distillate was titrated with standard acid 0.1 N HCL till pink colour has appeared. The per cent nitrogen was calculated as follows.

$$N_2 \text{ (per cent)} = \frac{[\text{Titre value of the sample} - \text{Titre value of the blank}] \times \text{Normality of HCL (0.1)} \times 14 \times 100}{\text{Weight of the sample} \times 1000}$$

The protein content was estimated in per cent by multiplying the obtained nitrogen per cent with factor 6.25 [8].

2.3.3 Crude fat of green gram during storage

Crude fat was determined by AOAC 922.06 – 2006 in Quality Control Laboratory, Rajendranagar, Hyderabad. Empty fat extraction beaker along with boiling stones (T) was weighed. Approximately two grams of the dehydrated seed was weighed in triplicates and packed into filter paper and placed in the thimbles provided with the instrument. Volume n – hexane taken in a beaker was recorded. Required conditions for Soxhlet extraction were maintained as per software settings and thimble containing seed sample was inserted into slot provided for thimbles. The thimble containing the test portion was immersed into the boiling solvent. The intermixing of the matrix with hot solvent ensured rapid solubilisation of

extractable. In the second step, the thimble was raised above the solvent and the test portion was further extracted by a continuous flow of condensed solvent for one hour 20 minutes. The solvent was evaporated and recovered by condensation. After drying, the resulting crude fat was determined gravimetrically. Extraction cups were dried at 102 ± 2 °C in hot air oven for 30 minutes to remove moisture. Excessive drying was avoided which can oxidize fat and give erroneous results. The defatted sample was cooled in a desiccator and weighed to the nearest 0.1 mg (F). The per cent crude fat was calculated as follows

$$\text{per cent Crude fat, Hexanes / equivalent extract} = \frac{F-T}{S} \times 100$$

Where,

F = Weight of cup + fat residue in grams

T = Weight of empty cup in grams

S = Test weight in grams

2.4 Statistical Analysis

The statistical methods described by Snedecor et al [9] were adopted in the present investigation. The data were subjected to angular transformation wherever necessary and two sample t- test was carried out to ascertain the results of effect of elevated levels of CO₂ on nutritional quality parameters by retesting the best treatment in comparison with untreated control in bulk seed.

3. RESULTS AND DISCUSSION

3.1 Effect of Modified Atmosphere with Elevated Levels of CO₂ on Carbohydrate Content of Green Gram Seeds, during Storage

The results pertaining to the effect of CO₂ treatment on carbohydrate content of stored green gram seeds are presented in Table 1. Initially, the carbohydrate content recorded was 62.96 per cent, with increase in storage duration the carbohydrate content was found to decrease in all the CO₂ treatments. After two months of storage, the lowest carbohydrate content was recorded in untreated control (60.20 per cent), which was found to be significantly inferior to the rest of the CO₂ treatments. The highest carbohydrate content (62.93per cent) was recorded in CO₂

treatment at 80 per cent after two months of storage.

The present investigation is in conformity with the findings of Shehata et al [10] who reported that under controlled atmosphere (up to 80 per cent CO₂), the stored cowpea seeds retained the highest total hydrolysable carbohydrate content. The initial carbohydrate content of green gram seed observed was 62.50 per cent. To ascertain the results, 80 per cent CO₂ treated green gram seeds (best treatment) were retested in bulk quantity (25 kg of seed) for further two months of treatment to test the performance in bulk and is compared its performance with the untreated control. From the results it can be concluded that the t-calculated value (24.70) is more than the t-table value (2.10) at 18 degrees of freedom and 5 per cent level of significance hence, the 80 per cent CO₂ treatment on carbohydrate content of green gram was significantly superior over the untreated control even in the bulk quantities (Table 2). Our results are in conformity with the findings of Ahmedani et al [11] who reported that reduction in carbohydrate was not solely dependent upon the progeny development. There are some other factors, which may have contributed towards reduction in carbohydrate contents. Such factors contribute varietal resistance as well as insect preference [12]. Results revealed that carbohydrate content decreased with *C. chinensis* infestation which might be due to depletion of cellulose and starch resulted from the metabolic and enzymatic activity of the insects as reported by Allali et al [13].

3.2 Effect of Modified Atmosphere with Elevated Levels of CO₂ on Total Protein of Green Gram Seeds during Storage

The results pertaining to the effect of CO₂ treatment on total protein content of stored green gram seeds are presented in Table 1. Initially, the protein content recorded was 21.56 per cent, with increase in storage duration it was found to increase in all the CO₂ treatments. The lowest protein content of 25.26 per cent was recorded in 80 per cent CO₂ treatment whereas, in untreated control 28.68 per cent of protein content was recorded and which was on par with 20 per cent CO₂ concentration (28.24 per cent). Gujar et al [14] reported that, initially the protein content in healthy green gram seed was 22.41 per cent which increased to 26.77 per cent due

Table 1. Effect of carbon dioxide (CO₂) treatment on nutritional parameters of green gram after two months of storage

S. No.	(CO ₂ Concentrations)	Carbohydrates (per cent)	Total protein (per cent)	Crude fat (per cent)
1	Carbon dioxide 20 per cent	60.57 (51.08)	28.24 (32.08)	4.16 (11.77)
2	Carbon dioxide 40 per cent	60.87 (51.26)	27.08 (31.35)	4.29 (11.95)
3	Carbon dioxide 60 per cent	61.45 (51.59)	26.20 (30.77)	4.40 (12.10)
4	Carbon dioxide 80 per cent	62.93 (52.15)	25.26 (30.16)	4.51 (12.25)
5	Untreated Control	60.20 (50.86)	28.68 (32.36)	3.98 (11.51)
	SE m±	0.12	0.15	0.04
	CD (P=0.05)	0.36	0.45	0.12
	CV (per cent)	0.42	0.86	0.57

The values in parentheses are angular transformed value

Table 2. Effect of carbon dioxide (CO₂) treatment on carbohydrate content of green gram (bulk quantity 25 kg) after two months of storage

t-Test: Two-Sample Assuming Equal Variances		
	CO ₂ (80 per cent)	Control
Mean	62.00	59.88
Variance	0.017	0.057
Df	18	
t Stat	24.70	
t Critical two-tail	2.10	

(Initial carbohydrate content: 62.50)

Table 3. Effect of carbon dioxide (CO₂) treatment on total protein content of green gram (bulk quantity 25 kg) after two months of storage

t-Test: Two-Sample Assuming Equal Variances		
	CO ₂ (80 per cent)	Control
Mean	26.03	28.71
Variance	0.018	0.03
Df	18	
t Stat	38.98	
t Critical two-tail	2.10	

(Initial total protein content: 21.50)

to *C. chinensis* infestation. The initial total protein content recorded in green gram seed was 21.50 per cent. From the results it can be concluded that the t- calculated value (38.98) is more than the t-table value (2.10) at 18 degrees of freedom and 5 per cent level of significance and hence, the 80 per cent CO₂ treated green gram sample on total protein content was significantly lowest over the untreated control even in the bulk quantities (Table 3). Present studies revealed that the total protein content increased with

increase in insect infestation, which is in conformity with the Bamaiyi et al [15], who reported total protein content increased by *C. maculatus* infestation in cowpea from one month to three months. Ahmedani et al [11] revealed that increase of total proteins is due to the addition of non-beneficial rather harmful proteins such as cast skins, exuviate, dead insects, wings, legs and other body parts of the insects that come along with the khapra beetle, *Trogoderma granarium* infested grain samples.

Table 4. Effect of carbon dioxide (CO₂) treatment on crude fat of green gram (bulk quantity 25 kg) after two months of storage

t-Test: Two-Sample Assuming Equal Variances		
	CO₂ (80 per cent)	Control
Mean	4.54	3.86
Variance	0.004	0.06
Df	18	
t Stat	20.75	
t Critical two-tail	2.10	

(Initial crude fat content: 4.66)

3.3 Effect of Modified Atmosphere with Elevated Levels of CO₂ on Crude fat of Green Gram Seeds during Storage

The results pertaining to the effect of CO₂ treatment on crude fat content of stored green gram seeds are presented in (Table 1). Initially, the crude fat content recorded was 4.66 per cent, with increase in storage duration it decreased in all the CO₂ treatments. The lowest crude fat content of 3.98 per cent was recorded in untreated control. The CO₂ treatment at 80 per cent recorded 4.51 per cent of crude fat content after two months of storage. From the results it can be concluded that the t-calculated value (20.75) is more than the t-table value (2.10) at 18 degrees of freedom and 5 per cent level of significance and hence, the 80 per cent CO₂ treated green gram sample on total crude fat content was significantly superior over the untreated control even in the bulk quantities (Table 4). The results with respect to crude fat, decreased with increased *C. chinensis* infestation. The present findings are in conformity with the Jood and Kapoor [16], who observed a significant decrease in fat content with the increase in infestation levels. Farhan et al [17] also reported a decline in maize fat content from 5.90 to 5.30 per cent after four months of storage due to damage by storage pests.

4. CONCLUSION

The study demonstrates that storing seeds in an 80 per cent CO₂ atmosphere effectively preserves high carbohydrate and crude fat content while slightly reducing protein levels after two months. This method, particularly when applied to 25 kg of seeds, was found to be the most effective in maintaining nutritional quality. While increased infestation by *Callosobruchus chinensis* negatively impacted carbohydrates and fat, it unexpectedly increased protein content in green gram seeds. Despite the challenges of

maintaining a sealed environment, CO₂ application offers a sustainable alternative to chemical pesticides, preserving grain integrity. While specific studies on green gram treated with elevated levels of carbon dioxide may be limited, research this topic can provide insights into the methodologies and considerations for such assessments. Further assessment of these silica powders should be carried out against various stored grain pests across different grain commodities, under varying temperature and humidity conditions, and using diverse application methods to effectively integrate them into management systems in storage arenas.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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