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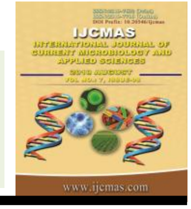

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# Nutritional Quality of Maize Kernel Artificially Infested by *Sitophilus oryzae* in Modified Atmosphere (MA) Storage with Elevated Levels of $CO_2$

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Nutritional quality of maize kernel artificially infested by *Sitophilus oryzae* and exposed to four different $CO_2$ concentrations *viz.*, 20, 40, 60 and 80 per cent in modified atmosphere was studied upto six months of storage. The results revealed that exposure of kernels to 80 per cent $CO_2$ concentration could able to maintain good per cent of carbohydrates (72.93 per cent), total proteins (8.67 per cent), crude fat (5.07 per cent) and crude fibre (2.40 per cent) upto six months of storage followed by 60 per cent $CO_2$ concentration. Whereas in 20 and 40 per cent $CO_2$ concentrations, per cent of carbohydrates, total proteins, crude fat and crude fibre losses was found to be more but these $CO_2$ concentrations could maintain good kernel quality upto two months of storage.

## Introduction

Maize occupies an important position in the world economy and traded as food and feed apart from its use as industrial crop for excellence. It serves as vital source of proteins, calories (in the form of carbohydrates and fats) and some of the important vitamins and minerals to billions of people worldwide particularly in Africa, South America and Asia and has been considered as poor man's nutricereal (Prasanna *et al.*, 2001).

*Sitophilus spp*. is an important post-harvest pest in maize. *Sitophilus oryzae* (Linnaeus) causes substantial losses in India and other Asian countries. *Sitophilus oryzae* is a major stored grain pest of rice and wheat but its polyphagous nature enables it to infect maize grain. Both the adults and the larvae of the weevils are internal feeders and can cause great losses to the grains both quantitatively and qualitatively. Grain loss between 12 and 20 per cent is common (Golob, 1984; Giga *et al.*, 1991) but upto 80 per cent has been reported in the untreated kernels (Mutiro *et al.*, 1992, Pingali and Pandey, 2001).

Wide use of insecticides for the control of stored grain insect pests is of global concern with respect to environmental hazards, insecticide resistance development, chemical residues in food, side effects on non-target organisms and the associated high costs (Cherry *et al.,* 2005). There are increasing restrictions on the use of pesticides and the number of chemical compounds officially registered for pest control in durable food products. Moreover, the use of methyl bromide for the fumigation of food commodities and facilities must be phased out in accordance with the Montreal protocol due to its effect on the ozone layer (UNEP, 2006). The development of alternative treatments for pest control is an increasing demand from the food industry and has been promoted by government through legislation and funding of research projects. Alternative treatments should meet consumer demand of reduced use or elimination of pesticides while at the same time maintaining a high degree of control efficacy.

Modified atmospheres (MA) have been used to disinfest raw or semi-processed food products such as cereal grains while still in storage. Treatments based on reduced oxygen ($O_2$) and high carbon dioxide ($CO_2$) or nitrogen ($N_2$) contents have been tested in laboratory and under industrial conditions. They are technically and economically suitable alternate fumigates for arthropod pest control in durable commodities in a number of countries (Fleurat – Lessard, 1990 and Adler *et al.,* 2000). Modified atmospheres (MAs) based on mixtures of gases with a high carbon dioxide ($CO_2$) content are known to be effective for the control of stored product pests that affect raw materials and semi processed and final food products (Adler *et al.,* 2000 and Navarro, 2006).

The use of $CO_2$ has several advantages, *viz*., there is no accumulation of toxic residues after the treatment in the final product and is considered as the safest traditional fumigant. Treatment with $CO_2$ is residue free and approved by Environmental Protection Agency (EPA), USA. $CO_2$ treated grains are also accepted in the organic market (Bera *et al.,* 2008).

## Materials and Methods

The present investigation on "Nutritional quality of maize kernel artificially infested by *Sitophilus oryzae* in modified atmosphere (MA) storage with elevated levels of $CO_2$" was conducted in the laboratory at Seed Research and Technology Centre, (SRTC), PJTSAU, Rajendranagar, Hyderabad, Telangana during 2017-2018. Nutritional quality analysis of maize kernel was carried out in MFPI-Quality Control Laboratory, PJTSAU and Central Instrumentation Cell, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad.

### Effect of modified atmosphere with elevated $CO_2$ as kernel protectant of maize during storage

To study the effect of modified atmosphere with elevated levels of $CO_2$, forty five air tight containers were filled with 500 g of disinfected maize seed and ten pairs of freshly emerged *S. oryzae* adults were released into the containers twenty five days prior to treatment with $CO_2$ to ensure a uniform level of infestation. Then, $CO_2$ was released at four concentrations *viz*., 20, 40, 60 and 80 per cent with three replications of each treatment. The required concentration of $CO_2$ was released into the container with a pressure of 2 kg $cm^{-2}$ from $CO_2$ cylinder. Before releasing the $CO_2$ into airtight container, the air present in the air tight container was flushed out by opening the outlet present at the top of the container and then it was closed with rubber cork and then the desired concentration of $CO_2$ was released into the airtight containers through the inlet located at the bottom of the containers by injecting the needle of $CO_2$ cylinder. After releasing the $CO_2$, the concentration of $CO_2$ was checked by using $CO_2/O_2$ analyzer (PBI 2006, Denmark). For determination of $CO_2$, the analyzer was calibrated with atmospheric air (20.9 % and 0.03% $CO_2$), then the needle of the analyzer was introduced into the top outlet tube of the air tight container and the measuring button of the $CO_2$ / $O_2$ analyzer was pressed. The concentration of $CO_2$ and $O_2$ present in the air tight containers was displayed on screen within 10 seconds which helped in determining the concentration of $CO_2$ present in the containers, then inlet and outlet tubes were closed at one stroke using rubber corks to prevent escape of $CO_2$ from the container.

After releasing the desired concentration into the containers, they were made air tight by plugging them with rubber corks and sealing with rubber tape.

Control was maintained by following the same procedure adopted for the $CO_2$ studies in plastic containers under laboratory conditions without exposing the seed to $CO_2$.

The air tight containers containing the disinfested seed exposed to different concentrations of $CO_2$ were observed at bimonthly intervals upto six months of storage. After completion of each exposure period, seal of the container was opened and observations on carbohydrates, total proteins, crude fat and crude fibre were recorded.

## Carbohydrates

Cereals are rich source of carbohydrates and contain 65-75 per cent of carbohydrates. The composition of carbohydrates differs in each cereal, starch being the main constituent. 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

## Total proteins

Maize kernels contain an average of 10-12 per cent protein. ‘Zein’ is the major protein of maize. Protein content was determined by available nitrogen in the sample by Kjeldhal method (AOAC, 1980). One gram sample was digested in 20 ml of sulphuric acid ($H_2SO_4$) at 420$^oC$ using copper sulphate and potassium sulphate as catalyst mixture. Digested sample was distilled using 40 per cent NaOH in KjelTech (CLASSIC-DXVATS (E)). Ammonia was absorbed in excess of four per cent boric acid solution and then titrated with standard acid (0.1N HCl) to estimate the nitrogen content.

$$\text{Nitrogen (per cent)} = \frac{\text{(Sample titre – Blank titre) X Normality of HCl X 14 X 100}}{\text{Weight of sample taken X 1000}}$$

The protein content was estimated using the following equation.

Protein (per cent) = 6.25 X Nitrogen (per cent)

## Crude fat

The whole maize kernel contains little amount of fat but germ is a rich source of oil. Uprety and Austin (1973) reported the fat content of 2.5 to 4.0 per cent in ten maize hybrids or composites grown in Delhi. Maize germ constitutes 5-14 per cent of the weight of kernel and is a good source of key nutrients especially 18-41 per cent of oil (Johnston *et al.,* 2005 and MPOC, 2008). Crude fat was determined by AOAC 2003.06 method in MFPI-Quality control laboratory, PJTSAU, Hyderabad.

## Crude fibre

The carbohydrates of cereals can be divided into available and unavailable carbohydrates. The available carbohydrates are those known to be hydrolysed by human alimentary enzymes. The remainder of the carbohydrates *i.e.* crude fibre (cellulose and lignin), hemicellulose and pectic substances which are not hydrolysed are grouped under unavailable carbohydrates. Uprety and Austin (1973) reported the crude fibre content ranging from 1.87 to 3.32 per cent in some maize hybrids and composites grown in Delhi. Crude fibre was determined by AOAC 962.09 method in MFPI-Quality control laboratory, PJTSAU, Hyderabad.

## Statistical analysis

The data was subjected to angular transformations wherever necessary and analysed by adopting Factorial Completely Randomized Design (FCRD) as suggested by Panse and Sukhatme (1978).

## Results and Discussion

### Carbohydrates of maize kernels under modified atmosphere with artificial infestation of *Sitophilus oryzae*

The carbohydrate content of maize kernels (Table 1 and Fig. 1) exposed to different concentrations of $CO_2$ did not show any significant variation among the treatments upto two months of treatment, while lowest carbohydrate content of 73.71 per cent was recorded in untreated control which was found to be significantly inferior to the rest of the treatments. Four months after treatment imposition, significantly highest carbohydrate content was recorded in 80 per cent $CO_2$(73.44 per cent) which was on par with 60 per cent $CO_2$ (73.33 per cent) while lowest carbohydrate content was recorded in untreated control (71.95 per cent). After six months of storage, significant differences among the treatments was found but highest carbohydrate content was recorded in 80 per cent $CO_2$ (72.93 per cent) followed by 60 per cent $CO_2$ (72.29 per cent), while lowest carbohydrate content was recorded in untreated control (68.61 per cent).

The results on mean carbohydrate per cent among the treatments varied from 72.40 to 73.50 per cent. The lowest mean carbohydrate content (71.40 per cent) was observed in untreated control followed by 20 per cent $CO_2$ (72.40 per cent).

While highest carbohydrate content (73.50 per cent) was recorded in 80 per cent $CO_2$ concentration which was significantly superior over other treatments. Results on different exposure periods revealed that mean carbohydrate per cent decreased with increase in exposure period.

The present investigations are in conformity with the findings of Shehata *et al.,* (2009), who reported that under controlled atmosphere (upto 80 per cent $CO_2$), the stored cowpea seeds retained the highest total hydrolysable carbohydrate. According to Chemeda Abedeta Garbaba *et al.,* (2017), carbohydrate content significantly decreased ($P < 0.05$) as the storage duration increased. Muhammad Shoaib Ahmedani *et al.,* (2009) also 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 are varietal resistance as well as insect preference.

### Total proteins of maize kernels under modified atmosphere with artificial infestation of *Sitophilus oryzae*

The total protein of maize kernels depicted in Table 1 and Figure 2 revealed that, after two months of treatment imposition, significantly highest total protein was recorded in untreated control (8.51 per cent) which is on par with the 20 per cent $CO_2$ concentration (8.48 per cent). Lowest total protein was recorded in 80 per cent $CO_2$ (8.21 per cent) concentration followed by 60 per cent CO2 concentration (8.27 per cent). After four months of treatment imposition, all the treatments were significantly different but lowest total protein was recorded in 80 per cent $CO_2$ (8.56 per cent) concentration followed by 60 per cent $CO_2$ (8.65 per cent) concentration. Significantly highest total protein content was recorded in untreated control (10.14 per cent). Similar trend was observed after six months of storage period, i.e., lowest total protein was recorded in 80 per cent $CO_2$ (8.67 per cent) concentration followed by 60 per cent $CO_2$ concentration (9.38 per cent) and significantly highest total protein content was recorded in untreated control (13.14 per cent). The interaction effect between the treatments and exposure periods with respect to total protein per cent showed that the least total protein content of 8.21 per cent was recorded after two months in 80 per cent $CO_2$ concentration and the highest total protein content of 13.14 per cent was recorded after six months of treatment in untreated control.

Present investigations revealed that the total protein content increased with increase in insect infestation which is in conformity with the Bamaiyi *et al.*, (2006) who reported that the percentage total protein determined by the estimation of total nitrogen content, showed an increase from one month to three months by *Callosobruchus maculatus* infestation in cow pea grains. Muhammad Shoaib Ahmedani *et al.*, (2009) revealed that an increase of total proteins is due to the addition of non-beneficial rather harmful proteins such as cast skins, exuviates, dead insects, wings, legs and other body parts of the insects that come along with the khapra beetle infested grain samples.

**Table.1** Effect of carbon dioxide ($CO_2$) treatment on carbohydrates and total protein of maize during different months of storage

| $CO_2$ Concentrations | Carbohydrates (%) | | | | Total Protein (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2MAT | 4MAT | 6MAT | Mean | 2MAT | 4MAT | 6MAT | Mean |
| 20 % $CO_2$ | 73.87 (59.26) | 72.85 (58.60) | 70.49 (57.10) | 72.4 (58.3) | 8.48 (16.93) | 9.55 (18.00) | 11.41 (19.74) | 9.81 (18.22) |
| 40 % $CO_2$ | 74.01 (59.35) | 73.05 (58.73) | 71.92 (58.00) | 73.0 (58.7) | 8.34 (16.78) | 9.14 (17.59) | 9.86 (18.30) | 9.11 (17.56) |
| 60 % $CO_2$ | 74.01 (59.35) | 73.33 (58.91) | 72.29 (58.24) | 73.2 (58.8) | 8.27 (16.72) | 8.65 (17.10) | 9.38 (17.83) | 8.77 (17.22) |
| 80 % $CO_2$ | 74.03 (59.36) | 73.44 (58.98) | 72.93 (58.65) | 73.5 (59.0) | 8.21 (16.65) | 8.56 (17.01) | 8.67 (17.12) | 8.48 (16.93) |
| Control | 73.71 (59.15) | 71.95 (58.02) | 68.61 (55.93) | 71.4 (57.7) | 8.51 (16.96) | 10.14 (18.57) | 13.14 (21.25) | 10.60 (18.93) |
| Mean | 73.93 (59.29) | 72.92 (58.65) | 71.25 (57.58) | | 8.36 (16.81) | 9.21 (17.66) | 10.49 (18.85) | |
| | SE(m)± | | CD (P=0.05) | | SE(m)± | | CD (P=0.05) | |
| Concentrations (F1) | 0.05 | | 0.15 | | 0.02 | | 0.05 | |
| Months after treatment (F2) | 0.04 | | 0.12 | | 0.01 | | 0.04 | |
| Interaction (F1*F2) | 0.09 | | 0.26 | | 0.03 | | 0.08 | |
| CV (%) | 0.26 | | | | 0.28 | | | |

Figures in parentheses are transformed values MAT- Months after treatment

**Table.2** Effect of carbon dioxide ($CO_2$) treatment on crude fat and crude fibre of maize during different months of storage

| $CO_2$ Concentrations | Crude Fat (%) | | | | Crude Fibre (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2MAT | 4MAT | 6MAT | Mean | 2MAT | 4MAT | 6MAT | Mean |
| 20 % $CO_2$ | 5.12 (13.07) | 4.98 (12.90) | 4.95 (12.85) | 5.02 (12.94) | 2.55 (9.29) | 2.61 (9.30) | 2.72 (9.49) | 2.63 (9.33) |
| 40 % $CO_2$ | 5.12 (13.07) | 5.00 (12.92) | 4.96 (12.86) | 5.02 (12.95) | 2.30 (8.72) | 2.52 (9.13) | 2.61 (9.29) | 2.47 (9.05) |
| 60 % $CO_2$ | 5.13 (13.09) | 5.08 (13.02) | 5.04 (12.97) | 5.08 (13.03) | 2.28 (8.79) | 2.44 (8.99) | 2.54 (9.28) | 2.42 (8.95) |
| 80 % $CO_2$ | 5.19 (13.17) | 5.11 (13.07) | 5.07 (13.02) | 5.12 (13.08) | 2.23 (8.60) | 2.28 (8.69) | 2.40 (8.91) | 2.30 (8.73) |
| Control | 4.91 (12.81) | 4.86 (12.74) | 4.70 (12.52) | 4.82 (12.69) | 2.63 (9.34) | 2.70 (9.56) | 2.84 (9.71) | 2.73 (9.50) |
| Mean | 5.09 (13.04) | 5.01 (12.93) | 4.94 (12.85) | | 2.40 (8.91) | 2.51 (9.11) | 2.62 (9.32) | |
| | SE(m)± | | CD (P=0.05) | | SE(m)± | | CD (P=0.05) | |
| Concentrations (F1) | 0.02 | | 0.04 | | 0.02 | | 0.05 | |
| Months after treatment (F2) | 0.01 | | 0.03 | | 0.01 | | 0.04 | |
| Interaction (F1*F2) | 0.03 | | 0.08 | | 0.03 | | 0.08 | |
| CV (%) | 0.36 | | | | 0.55 | | | |

Figures in parentheses are transformed values MAT- Months after treatment

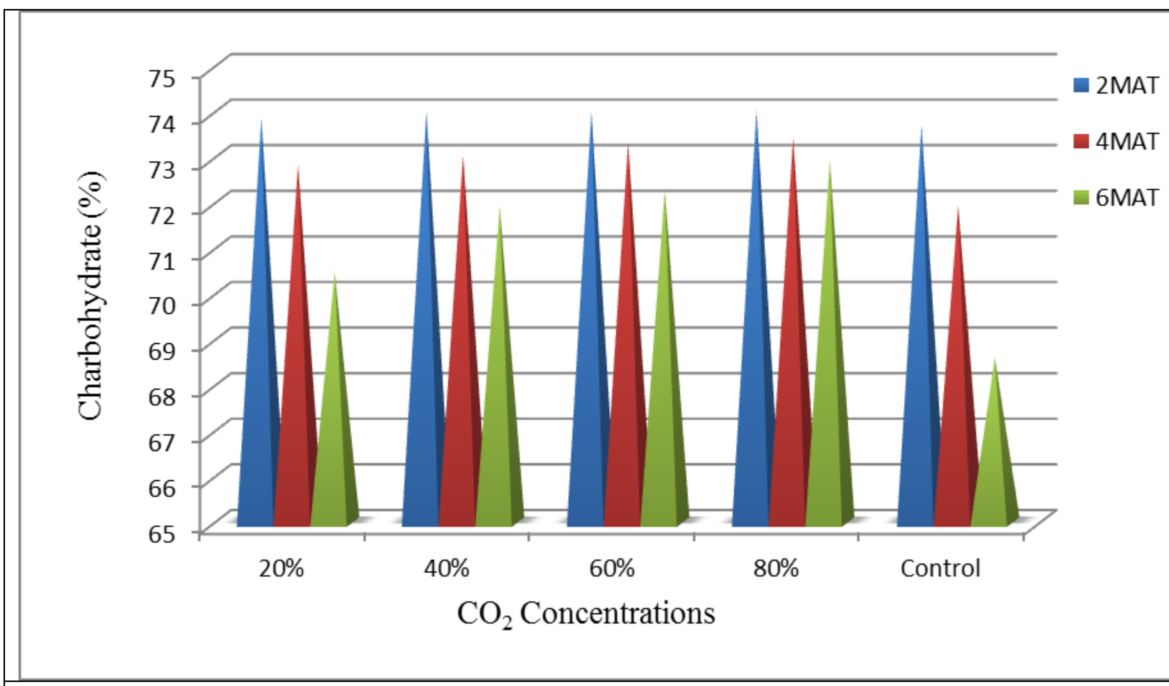


**Fig.1** Effect of elevated levels of $CO_2$ on carbohydrates of maize kernels

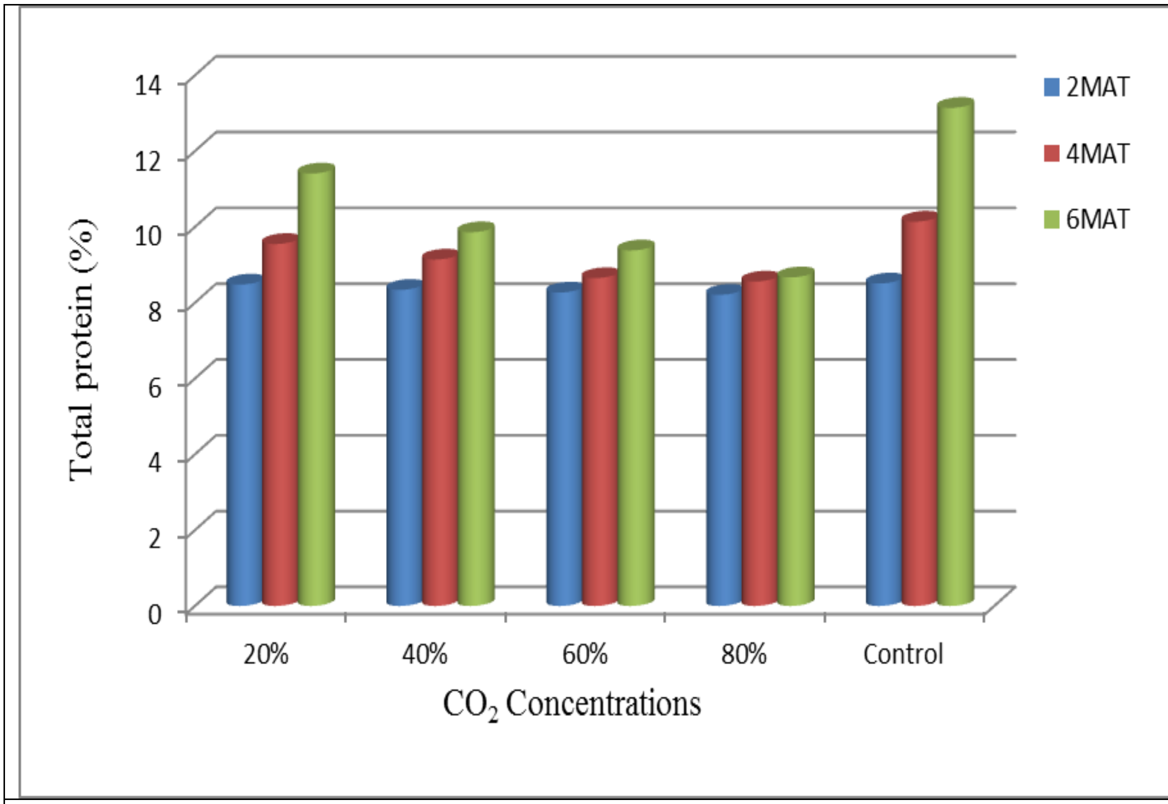


**Fig.2** Effect of elevated levels of $CO_2$ on total proteins of maize kernels

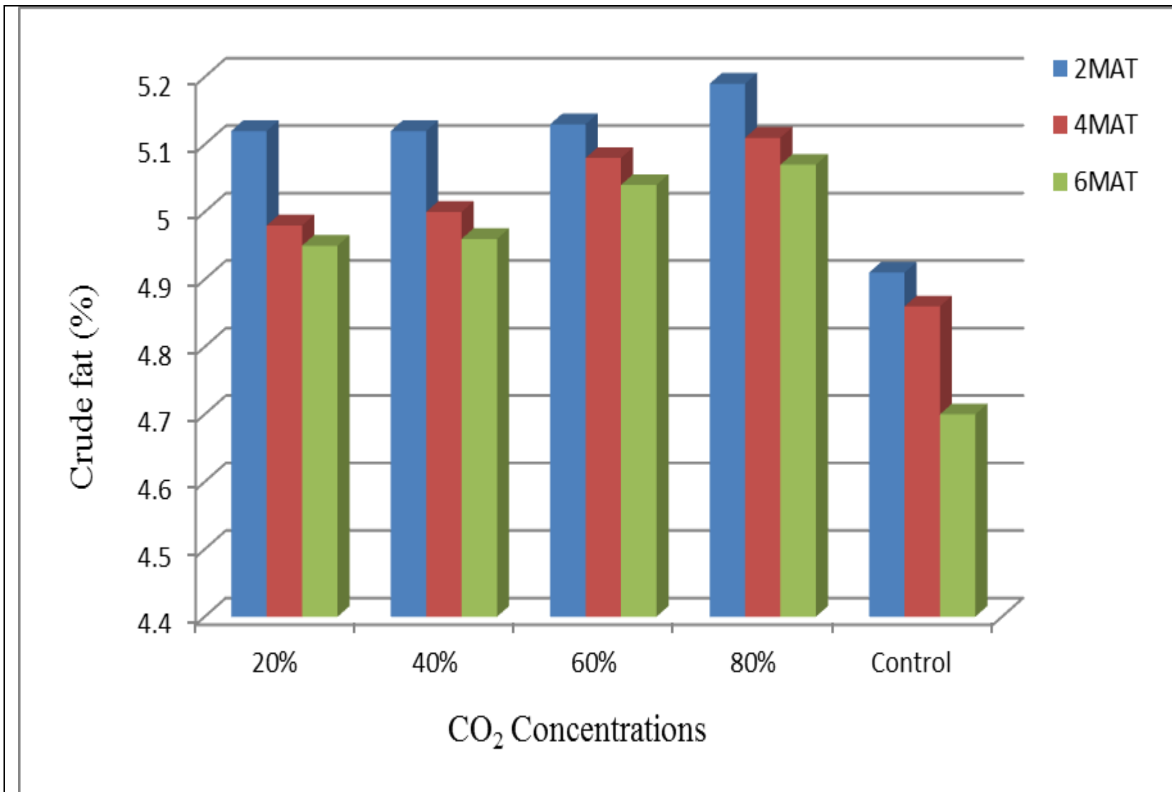


**Fig.3** Effect of elevated levels of $CO_2$ on crude fat of maize kernels

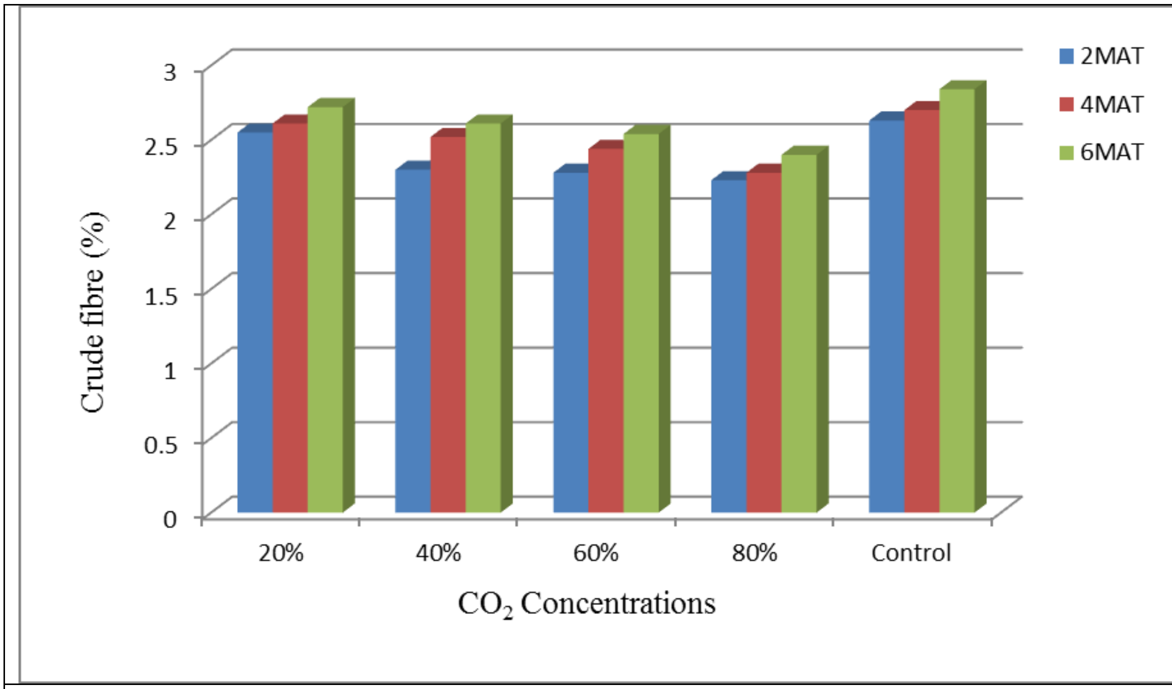


**Fig.4** Effect of elevated levels of $CO_2$ on crude fibre of maize kernels

**Crude fat of maize kernels under modified atmosphere with artificial infestation of *Sitophilus oryzae***

The data with regard to crude fat of maize kernels (Table 2 and Fig. 3) showed that, after two months of treatment imposition, significantly lowest crude fat was recorded in untreated control (4.91 per cent). Highest crude fat was recorded in 80 per cent $CO_2$ concentration (5.19 per cent) followed by 60 per cent $CO_2$ concentration (5.13 per cent).

After four months of treatment imposition, significantly lowest crude fat was recorded in untreated control (4.86 per cent). Highest crude fat was recorded in 80 per cent $CO_2$ concentration (5.11 per cent) which is on par with 60 per cent $CO_2$ concentration (5.08 per cent). Similarly after six months of storage period, highest crude fat was recorded in 80 per cent $CO_2$ concentration (5.07 per cent) which is on par with 60 per cent CO2 concentration (5.04 per cent) and significantly lowest crude fat content was recorded in untreated control (4.70 per cent).

The results on mean crude fat per cent among the treatments varied from 4.82 to 5.12 per cent. The highest mean crude fat content (5.12 per cent) was observed in 80 per cent $CO_2$ which is on par with 60 per cent $CO_2$ (5.08 per cent). While lowest crude fat content (4.82 per cent) was recorded in untreated control. The results with respect to crude fat decreased as storage period increased.

The present investigations are in conformity with the findings of Jood and Kapoor (1993) who observed a significant decrease in fat content with the increase in infestation levels. Samuels and Modgil (2003) also reported significant decrease in crude fat with increase in insect infestation and storage period.

Farhan *et al.,* (2013) also reported a decline in maize fat content from 5.9 per cent to 5.3 per cent after four months of storage due to damage by storage pests. Similarly, Stefanello *et al.,* (2015) has also reported that fat content declined from 5.8 per cent to 5.0 per cent after nine months of storage in plastic bags at room temperature.

### Crude fibre of maize kernels under modified atmosphere with artificial infestation of *Sitophilus oryzae*

The data pertaining to crude fibre of maize kernels (Table 2 and Fig. 4) showed that, after two months of treatment imposition, significantly lowest crude fibre was recorded in 80 per cent $CO_2$ concentration (2.23 per cent) which is on par with 60 per cent $CO_2$ (2.28 per cent) concentration. Highest crude fibre was recorded in untreated control (2.63 per cent). After four months of treatment imposition, all treatments significantly varied but lowest crude fibre was recorded in 80 per cent $CO_2$ concentration (2.28 per cent) followed by 60 per cent $CO_2$ concentration (2.44 per cent). Significantly highest crude fibre content was recorded in untreated control (2.70 per cent). Similarly after six months of storage period, lowest crude fibre was recorded in 80 per cent $CO_2$ concentration (2.40 per cent) followed by 60 per cent $CO_2$ concentration (2.54 per cent) and significantly highest crude fibre content was recorded in untreated control (2.84 per cent).

The interaction effect between the treatments and exposure periods with respect to crude fibre per cent showed that the least crude fibre content of 2.23 per cent was recorded after two months in 80 per cent $CO_2$ concentration and the highest crude fibre content of 2.84 per cent was recorded after six months of treatment in untreated control.

Present investigations revealed that the crude fibre content increased with increase in insect infestation which is in conformity with the Srivastava *et al.,* (1988) who observed increase in crude fibre with storage time in pigeonpea. The observed increase in crude fibre might be due to increased infestation with a decrease in non-fiber constituents like starch and soluble carbohydrates. Muhammad Shoaib Ahmedani *et al.,* (2009) reported that crude fibre contents generally increased after infestation on overall weight basis and they confirmed the mean increase from 2.05 to 3.04 per cent while working on varietal changes in nutritional composition of a wheat kernel caused by Khapra beetle infestation. Fibre content significantly increased as storage duration increased. Bamaiyi *et al.,* (2006) also reported that the percentage fibre was higher in the *Callosobruchus maculatus* infested grains than in the control and it increased with infestation period.

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