J. Appl. Biosci., 50(2): 171-178, December, 2024
doi: 10.61081/joab/50v2i114

ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

ORIGINAL ARTICLE

Influences on the Kernel Quality of Maize under Elevated Carbon Dioxide Exposure

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ABSTRACT

Anthropogenic activities have increased atmospheric carbon dioxide (CO_2) levels for the past few decades and continue to increase. Carbon dioxide is a greenhouse gas and escalating CO_2 has a far-reaching impact on agroecosystem productivity. Physiological features like stomatal conductance, photosynthesis rate, RuBisCO carboxylation efficiency, and growth attributes typically show an improvement along with alterations found in the seed quality of major crops. The present study assessed the influences of elevated CO_2 on the kernel quality of a maize cultivar DHM117 using OTCs under field conditions. The setup included ambient CO_2 [ACO_2] which served as control and elevated CO_2 [ECO_2] (700 ppm±30). The quality analyses showed noteworthy changes in various nutritional parameters under elevated levels of CO_2. Total soluble sugars and starch contents in grains increased due to ECO_2. In contrast, protein, total free amino acids and essential amino acids like tryptophan and lysine content declined in the grains of ECO_2. Moreover, many nutrient elements like Na, K, Cu, Fe, Ni, and Zn were found to be reduced under CO_2 enrichment while Ca, Mg, and Mn values declined. Elevated CO_2 treatments resulted in the unsaturation of oil with a reduction in saturated fatty acid content. Hence elevated CO_2 enhanced the starch value in maize kernel which is a positive attribute for a cereal plant, however, a reduction in essential amino acids tryptophan, lysine and a few nutrient elements will compromise the nutritional value of maize.

KEY WORDS : Amino acid, Carbon dioxide, Fatty acid, Maize, Nutrient elements, Quality

INTRODUCTION

Since the Industrial Revolution, the World has witnessed an escalation in atmospheric carbon dioxide (CO_2) concentrations which is a significant ecological transformation (Peters *et al.*, 2020). Carbon dioxide is a greenhouse gas (GHG) and its abundance leads to the phenomenon of global warming and climate change (Maurya *et al.*, 2020). Since the dawn of the Industrial Revolution, levels of atmospheric CO_2 have escalated from 280 ppm to 412 ppm (Naqvi *et al.*, 2020). Carbon dioxide is utilized by plants during photosynthesis to make sugars for energy metabolism or may be used as the raw material for the production of cellulose and starch, proteins or other organic compounds essential for growth and development. Direct influences of enhanced CO_2 levels are typically advantageous, such as photosynthesis stimulation, improved water use efficiency, reduced stomatal conductance, and enhanced carbon allocation to the roots (Reddy *et al.*, 2010; Xu *et al.*, 2015; Mishra *et al.*, 2024). Hence, apart from their influences on climate, CO_2 enrichment directly influences the plant life resulting in an enhancement in growth and yield (Ainsworth *et al.*, 2020; Singh *et al.*, 2023). Doubling of atmospheric CO_2 concentration from 340 to 680 ppm would upsurge the yield of economically important crops by 10 to 15% (Ainsworth *et al.* 2006). When averaged across the 18 soybean genotypes, 550 ppm elevated CO_2 increased the seed yield by 22% (Bishop *et al.*, 2014).

Apart from seed yield, CO_2 enrichment also affects the seed quality. Upreti *et al.* (2007) found that CO_2 enrichment significantly modified the quality of rice grains in Pusa Sugandh-2 and Pusa rice hybrid varieties. Approximately 29% more sucrose accumulation in sugarcane plants was detected in the plants growing under 380 and 740 ppm CO_2 (De Souza *et al.*, 2008). A meta-analysis by Taub *et al.* (2008) detected that crop plants had reduced protein values when grown at elevated CO_2 (540-958 ppm) as compared to ambient CO_2 (315-400

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ppm) in various crop plants like rice, wheat, soybean, barley, and others. The mineral content (macro and microelements) in crop grains is usually reduced by the elevated CO_2 which decreases their nutritional value (Loladze, 2002). Carbon dioxide enrichment resulted in an alteration in the macro and microelements concentrations reducing the elements like Ca, Fe, K, N, P, Mg, S, and Zn, in wheat grains due to the dilution effect, caused by the increased carbohydrates concentration in the seeds (Upreti *et al.*, 2010). Grain nutrient concentration for the elements Fe, Cu, Zn, Ca, Mg, Na, S, and P in cultivars Janz and Yitpi was significantly reduced by elevated CO_2 treatment (Fernando *et al.*, 2014). Like the grain nutritional value, Pal *et al.* (2014) found that the fatty acid composition of sunflower oil had a higher proportion of unsaturated fatty acids (oleic and linoleic acid) under elevated CO_2 (550±50 ppm) environment.

Zea mays L. or maize is the third significant staple crop across the world, and fifth in India for its contribution to the global maize economy (Singh *et al.,* 2018). Maize is the source of food and feed and its demand will increase continuously because of increasing demand in livestock and poultry sectors in the country. The farming and yield of C4 crops like maize, sugarcane, and sorghum are impacted by various environmental aspects. Hence, the influences of high CO_2 concentration in the atmosphere have been researched to explore the growth and development of C4 plants (Da Silva *et al.*, 2020). However, investigations related to CO_2 effects on maize seed quality are limited. Therefore, considering the importance of a C4 plant maize in terms of its nutritional value, an assessment of the CO_2 fertilization effect on maize plants against elevated CO_2 on kernel nutrient quality has been done in the present experiment.

MATERIAL AND METHODS

Experimental Setup and Plant Material

The experiment was conducted between December 2013 to April 2014 at the Agriculture Research Farm of Banaras Hindu University, Varanasi located at 25° 14' N latitude and 82° 03' E longitude at 76.1 m above the mean sea level. *Zea mays* L. commonly known as corn or maize was selected as the model plant and was grown in open-top chambers according to the design of Bell and Ashmore (1986). The chambers with ambient CO_2 concentration were designated as ACO_2 and the ECO_2 treatments were supplied with elevated CO_2 concentration of 700 ±30 ppm. Carbon dioxide concentration was standardized by employing the automatic CO_2 analyzer (Li-COR Inc., USA). Field preparation and kernel sowing was done according to the standard agricultural practices in which plant-to-plant distance was maintained at 20 cm and the distance between the rows was kept at 60 cm. Recommended fertilizer doses were added as 120, 60, and 40 kg/ha NPK, respectively and uniform irrigation of soil was followed. Elevated CO_2 supply was given 12 days after the plant emergence. The treatment of carbon dioxide was decided according to the predictions of IPCC (2007), and its dose was provided with the help of CO_2 cylinders equipped with regulated gas flow. The concentration of CO_2 was monitored by an automatic CO_2 analyzer (Li-COR Inc., USA). After the complete maturity of the plants, harvesting was done and cobs were collected. Maize kernels were separated and dried for further experimentation of kernel quality analysis.

Kernel Quality Analysis

Maize kernels were grounded to make a fine powder and passed through a sieve of 2 mm mesh size in a stainless-steel grinder. The kernels were analyzed for carbohydrate determination like reducing sugar, total soluble sugar, and starch. Further, estimations were also done for nitrogen, phosphorus, nutrient elements, protein, amino acid, and oil quality. Maize oil was also analyzed for fatty acid profiling and oil quality parameters.

Carbohydrate, Protein, Total Free Amino acid, Tryptophan and Lysine

Analysis for carbohydrates was done by following the methodology proposed by Somogyi-Nelson Herbart *et al.* (1971) for reducing sugar and Dubois *et al.* (1956) for starch and total soluble sugars. Excised endosperm (i.e. degermed kernel) was utilized for nitrogen, protein, tryptophan, and lysine determination. Protein was determined by the method of Lowry *et al.* (1951). The total free amino acid was assayed by the method of Moor and Stein (1948). Tryptophan and lysine contents were estimated following the procedure illustrated in Villegas *et al.* (1984).

Nutrient Elements

Nitrogen values in the samples were measured by a Gerhardt automatic analyzer (Model KB8S, Germany). Kernel phosphorous content was assessed by the methodology of Jackson (1958). For nutrient elements (macro and trace) estimation, 0.1 g of grounded maize flour was passed through a 2 mm sieve and was digested in a mix of 9 parts HNO_3 and 4 parts H_2SO_4 diacid by the procedure of Allen *et al.* (1986). Samples undergoing digestion were filtered through whatmann no. 42 filter paper and distilled water were utilized to make up the volume up to 25 ml. Nutrient elements like Cu, Mg, Mn, Fe, Ni, and Zn were determined using an atomic absorption spectrophotometer (Model 2380, Perkin Elmer, New Jersey, USA). Ca, K, and Na concentrations were determined using a flame photometer (Systronics Flame photometer 128, Ahmedabad, India).

Extraction of Oil and Analysis of Oil Quality

Grounded kernels weighing 200 g from ACO_2 and ECO_2 treatments were filled in paper thimbles and then placed in a soxhlet extractor fitted with a 100 ml round bottom flask. The extracting solvent was petroleum ether and six cycles were repeated. The solvent was distilled off in a rotary evaporator at 60°C temperature under vacuum, post-completion of oil extraction. Oil volume was measured and the fatty acid profile was examined as per the standard method of BIS: 548-(P-III)-(1976). Oil quality data is expressed as mean values of fatty acid percentage out of the total fatty acids detected in the oil sample. Estimation of iodine value (oil unsaturation) and saponification value (a measure of average molecular weight of all the fatty acids) of oil was done following the standard methods of BIS: 548-(P-I)-(1964).

Statistical Analysis

There were nine replicate samples for each treatment for the carbohydrate parameters, protein, TFAA, and nutrient elements, and oil quality analysis was done in triplicates. All the data sets were analysed for significance using the T-Test analysis and statistical analyses were performed using SPSS software (SPSS Inc. version 16.0).

RESULTS

Carbon dioxide concentration

Daytime mean ambient CO_2 concentration ranged from 377.5 to 402.0 ppm from December 2013 to April 2014 (Fig. 1) and the mean CO_2 value for the experimental period was 391.9 ppm.

Table 1: Effect of the treatments ($ACO_2$and ECO_2) on nutrient elements of DHM117 cultivar. Values are mean ± SE. Levels of significance are: *NS* non-significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Nutrients	Treatments	
	ACO_2	ECO_2
Phosphorus (mg g^{-1})	3.02 ± 0.03	3.32 ± 0.02^{NS}
Potassium (mg g^{-1})	2.66 ± 0.07	2.46 ± 0.06**
Sodium (mg g^{-1})	2.09 ± 0.05	1.33 ± 0.17***
Calcium (mg g^{-1})	1.42 ± 0.03	1.72 ± 0.11***
Magnesium (mg g^{-1})	1.33 ± 0.23	1.80 ± 0.07***
Zinc (µg g^{-1})	54.0 ± 0.19	30.0 ± 0.54***
Copper (µg g^{-1})	2.71 ± 0.51	2.0 ± 0.02***
Manganese (µg g^{-1})	2.50 ± 0.04	5.73 ± 0.15***
Iron (mg g^{-1})	4.81 ± 0.05	4.18 ± 0.05***
Nickel (µg g^{-1})	27.25 ± 0.002	24.0 ± 0.005***

Carbohydrate, protein, total free amino acids, tryptophan, and lysine content in kernels

Starch content and total soluble sugar increased under ECO_2 as compared to ACO_2. Starch values were increased under ECO_2 by 12.66% compared to ACO_2, while, under ECO_2 environment, reducing sugar manifested a significant decline of 22.47% (Fig. 2). Moreover, soluble protein content in maize kernels was reduced under ECO_2 treatment as compared to ACO_2. A respective decline of 7.30% in total protein and 8.59% in TFAA content was observed under ECO_2 treatment compared to the ACO_2 environment (Fig. 2). Nitrogen in maize kernels was decreased under ECO_2 by 20.87% compared to ACO_2 (Fig. 2). The essential amino acids lysine and tryptophan showed reductions under elevated CO_2 exposure as compared to ACO_2 (Fig. 2).

Nutrient Elements

A significant increase in P by 9.92% under ECO_2 was observed with respect to ACO_2. While under ECO_2, the decline in Na and K content was approximately 35.94 and 7.52%, respectively as compared to ACO_2 (Table 1). Ca and Mg were enhanced under ECO_2 treatment as compared to ACO_2. Mg values were increased by 35.32% as compared to ECO_2 while Ca was increased by 21.13% in ECO_2 treatment as compared to ACO_2 (Table 1). Zn and Cu displayed respective reductions of 44.52 and 25.89% under ECO_2 as compared to ACO_2. However, a substantial increase of 129.17% was observed for Mn under ECO_2 compared to ACO_2. Both Fe and Ni declined in elevated CO_2 atmosphere compared to ACO_2. Under ECO_2, respective percent reductions recorded in Fe and Ni were 13.14 and 11.89% compared to ACO_2 (Table 1).

Oil Quality Parameters

Elevated CO_2 led to unsaturation of oil (Fig. 4). Saturated fatty acid declined by 16.45% under ECO_2 (Fig. 4). Percentage increase in oil unsaturation was 1.54% in ECO_2 concerning ACO_2. A reduction in saturated fatty acids was detected under ECO_2 leading to an absence of saturated fatty acids like arachidic and lignoceric acid (Fig. 3). A decrease in eicosanoic acid was found under ECO_2 by 79.54% (Fig. 4). Saturated fatty acids like palmitic acid and stearic acid were reduced due to CO_2 treatment with respect to ACO_2.

Linolenic acid/omega 3 fatty acid increased by 53.33% under ECO_2 compared to ACO_2 treatment (Fig. 3). A minor reduction of 9.64% was observed in Linoleic acid/omega 6 fatty acids under ECO_2 compared to ACO_2. Maize oil consists of a major composition of oleic acid/omega 9 fatty acid which is enhanced under ECO_2 compared to ACO_2. Three new unsaturated fatty acids (eicosadienioic acid, erucic acid, and docosadienioic acid) appeared under ECO_2treatment which was not detected under ACO_2.

Monounsaturated fatty acid increased by 19.16 % at ECO_2 compared to ACO_2. Polyunsaturated fatty acids were decreased under ECO_2 treatment by 13.01% compared to ACO_2 (Fig. 3). Iodine value displayed reduction under ECO_2 treatment as compared to ACO_2. Further, the Saponification value increased under ECO_2 with respect to ACO_2 (Fig. 3).

DISCUSSION

Alterations in crop quality, such as nutritional value, are of prime significance for the evaluation of climate change components like CO_2 influences on future food productivity (Broberg *et al.,* 2017). Hogy & Fangmeier (2008) have detected that elevated CO_2 resulted in higher starch values in wheat grains suggesting an increase in carbohydrate translocation from the source (leaves and stem) to sink (grain). Poorter *et al.* (1997) viewed that the enhanced starch concentration under elevated CO_2 is due to the increase in photosynthetic rate. The significant increase in total sugars, reducing, non-reducing, and starch content in grains under CO_2 enrichment eventually increased the grain mass in wheat plants (Upreti, *et al.,* 2007).

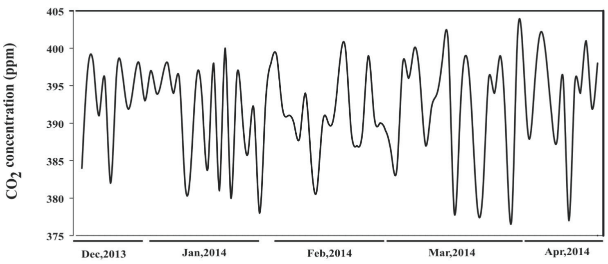

Fig. 1. Daily mean (10h) CO_2 concentration for the period December 2013-April 2014 at the experimental site.

Carbon dioxide enrichment has been found to reduce the total protein content (Taub *et al.*, 2008; Yadav *et al.*, 2021). *Triticum aestivum* cultivars K-9107 and HUW-37 displayed respective significant reductions of 10.4, 6.9, and 8.9, 5.4% for protein content and TFAA in grains under elevated CO_2 treatment (Mishra *et al.*, 2013). Similarly, under elevated CO_2 environment, reductions in nearly all the individual and total amino acids were reported (Hogy *et al.*, 2009). In the present experiment, N also followed a similar trend to that of protein which significantly reduced under CO_2 enrichment. A decline in seed N content in maize under ECO_2 has also been reported by Abebe *et al.* (2016). This decline in N content is attributed to CO_2-induced limitation of transpiration due to partial stomatal closure (McDonald *et al.*, 2002), leading to reduced N uptake and dilution of N under the condition of more carbohydrate production or alterations in the nitrogen allocation (Fangmeier *et al.*, 2000; Broberg *et al.*, 2017). Tryptophan and lysine content of the maize kernels reduced under CO_2 in the present investigation although they both are essential amino acids. A similar reduction in the lysine content in maize kernels has been observed by Yadav *et al.* (2021).

Under ECO_2, Na, K, Ni, Cu, and Zn concentrations in kernels were lower than ACO_2. An increase in the amount

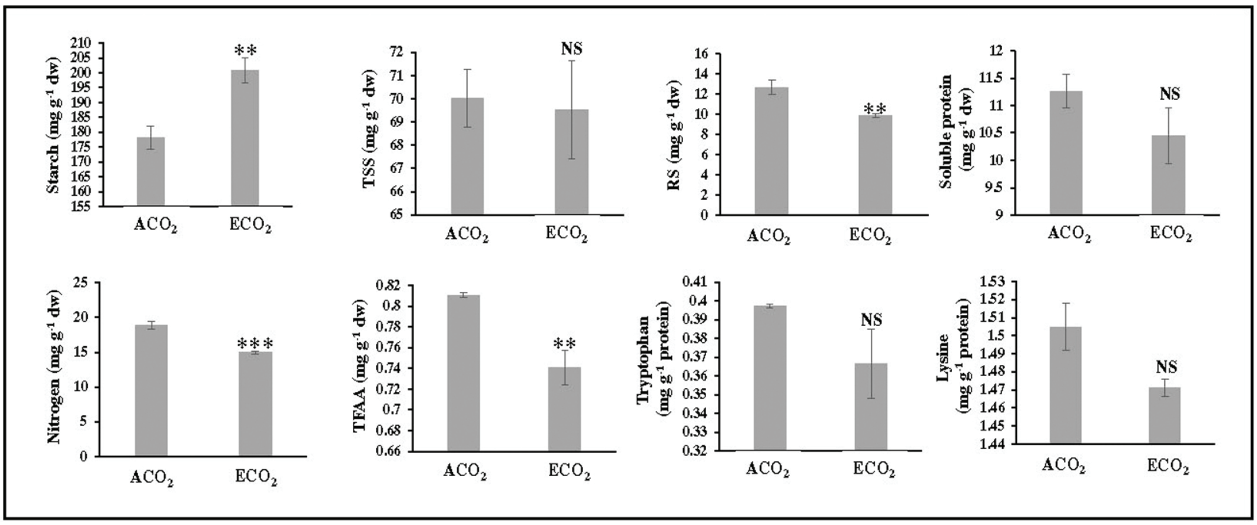

Fig. 2. Grain quality parameters (starch, total soluble sugar, reducing sugar, soluble protein, nitrogen, total free amino acid, tryptophan and lysine) in ACO_2 and ECO_2 treatments; Values are mean ± SE. Levels of significance are: *NS* non-significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

of photosynthates accumulated in leaves might have produced the dilution effect in maize kernels resulting in reduced nutrient concentrations. Hogy & Fangmeier (2009) have suggested that a decline in mineral nutrients under elevated CO_2 is most likely because of a dilution effect due to the enhancement of dry matter. However, increments were observed in P, Ca, Mg, and Mn under ECO_2 compared to ACO_2. Pal *et al.* (2005) found that most of the micronutrients such as Ca, Cu, Fe, Na, K, Mn, and Zn reduced under elevated CO_2 treatment except P. Similarly, a reduction in Ca, Fe, Mg, S, and Zn content was observed in seeds obtained from mustard plants grown under elevated CO_2 treatment over the NF control (Singh *et al.*, 2013) Abebe *et al.* (2016) have detected that P content declined by 11% but K content increased by 5% in maize grains under elevated CO_2 over the ambient CO_2. The majority of the phosphorus in cereal grains binds ions such

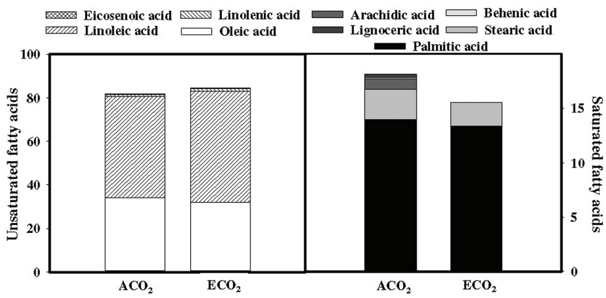

Fig. 3. Fatty acid profile of DHM117 cultivar under ambient CO_2(ACO_2) and CO_2 (ECO_2) enrichment to show the respective unsaturated and saturated fatty acid percentage.

as Mg (Batten, 1994), which usually explains the strong correlation between P and Mg concentrations in grains.

There are significant effects of CO_2 on the fatty acid composition of oil. The enhancement in seed oil content is possibly at the cost of either protein or carbohydrate. An increase in oil content was found under ECO_2 compared to ACO_2 treatment. According to Upreti *et al.* (1996), an increase in oil volume can be attributed to the involvement of additional CO_2 in stimulating the acetyl CoA enzyme activity which enhances the formation of abundant malonyl CoA under elevated CO_2 and acts to regulate the fatty acid biosynthesis positively. Elevated CO_2 treatment led to unsaturation of oil with corresponding reductions in the composition of saturated fatty acids. Depreciation in stearic, palmitic, and behenic acids were found under elevated CO_2. Supporting our results, elevated CO_2 altered the fatty acid composition of *Brassica juncea* seeds and it was found that elevated CO_2 brought about a reduction in saturated fatty acids (palmitic and stearic acid) signifying that the majority of the fatty acids undergo desaturation and produce unsaturated fatty acids due to lower O_2:CO_2 ratio (Uprety *et al.*, 2007).

Oleic acid (omega-9 fatty acid) was found to be increased under ECO_2 treatment. The percentage of linolenic acid (omega-3 fatty acid) was increased in ECO_2 which could be due to the conversion of linoleic acid to linolenic acid (Covington, 2004). Linolenic acid or omega-3 fatty acids in human diets are usually associated with a reduction in cardiovascular diseases and act as anti-inflammatory and anti-thrombotic (Covington, 2004). Carbon dioxide enrichment led to an increase in the

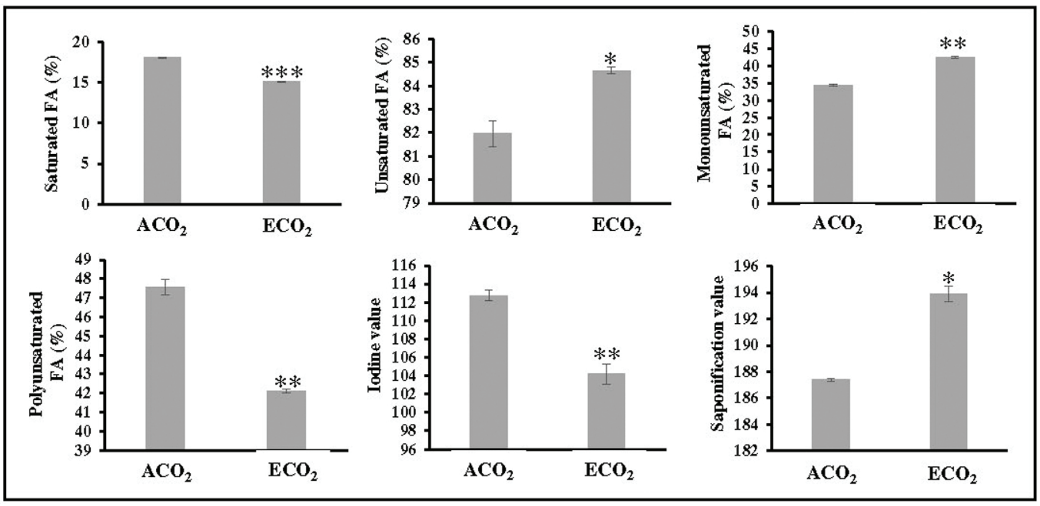

Fig. 4. Oil quality parameters in ACO_2 and ECO_2 treatments. Values are mean ± SE. Levels of significance are: *NS* non-significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

percentage of MUFA and simultaneous reduction in PUFA and this change is positive for maize oil composition, since oils with higher magnitudes of unsaturated fatty acids are more prone to auto-oxidation than those containing, lesser proportions (Tan & Man, 1999; Tripathi & Agrawal, 2012) as well as a diet rich in MUFA is known to reduce the cholesterol level in blood (Delpanque, 2000) and reduces coronary heart disease (Grundy, 1986). Three new unsaturated fatty acids (eicosadienioic acid, erucic acid, and docosadienioic acid) were detected under ECO_2 treatment possibly due to the availability of more photosynthates under CO_2 enrichment leading to the production of new fatty acids which were not detected under ACO_2 treatment. While the appearance of erucic acid was advantageous since low erucic acid content is needed for good human health (Wang *et al.*, 2010). Elevated CO_2 reduced the palmitic acid by 3% whereas oleic acid was increased by 4% (Burkey *et al.*, 2007). The increase in oleic acid at elevated CO_2 was linked with a decline in linoleic acid to the same extent (Burkey *et al.*, 2007) and similar results have been observed in the present study also.

The iodine value decreased in the ECO_2 atmosphere while the saponification value was enhanced with respect to ACO_2. The higher iodine value indicated higher degree of unsaturation. An increase in iodine value indicate enhancement in reactivity, less stability, and rancidification of oil (Tripathi & Agrawal, 2012); so, a drop in the iodine value under CO_2 enrichment is a beneficial effect. The saponification value is an estimation of the average molecular weight or chain length of all the fatty acids present in oil. High saponification value shows the presence of short-chain fatty acids because they have a greater number of carboxylic acid functional groups per unit mass as compared to long-chain fatty acids hence it was found that CO_2 elevation favoured the synthesis of short-chain fatty acid with a greater number of carboxylic acid groups which usually protects the oil against rancidity.

CONCLUSIONS

Carbon dioxide is the most significant greenhouse gas that is emitted from man-made sources in terms of amount and effects on climate. Anthropogenic activities like the burning of fossil fuel and deforestation are accountable for the sizable rise in CO_2 and project to continue in the future. Alterations in the kernel quality of maize due to elevated CO_2 were observed. Carbon dioxide enrichment to plants induced the starch accumulation in kernels with a drop in nitrogen and soluble protein. Several nutrient elements were found to be reduced under CO_2 elevation. ECO_2 treatments led to oil unsaturation with depreciation in saturated fatty acids while enhancement in unsaturated essential fatty acids likes linolenic acid. Oil got more unsaturated under elevated CO_2 treatment due to the appearance of three new unsaturated fatty acids i.e. erucic acid, docosadienioic acid, and eicosadienioic acid. Hence CO_2 elevation induced the carbohydrate accretion but reduced the nitrogen and proteins and also decreased the mineral nutrition value of the maize kernels.

Acknowledgements

SBA is thankful to CSIR, New Delhi for providing "Emeritus Scientist Fellowship".

Conflict of Interest

Authors declare no conflict of interest.

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