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Chemical Analysis of BT corn "Mon-810: Ajeeb-YG®" and its counterpart non-Bt corn "Ajeeb"

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Abstract: *Commercialization of biotech crops has started since 1996, where the cultivated area of these crops was increased from 1.7 million hectares in 1996 to 170.3 million hectares in 2012 according to the latest statistics in 2012. Bt corn "MON810: Ajeeb YG®" is one of these crops that express endotoxin from Bacillus thuringiensis (Bt) throughout the whole plant. This study was designed to assess the safety of Bt corn by comparing its compositional chemical analysis with its conventional counterpart "Ajeeb". Moisture content, crude fat, total saccharides, starch & crude fiber were determined; sodium, potassium, magnesium, calcium and phosphorous content were measured, tannins & phytic acid were determined as anti-nutrients. Amino acids and fatty acids profiles were also evaluated. Results indicated the presence of significant differences between both of Bt corn and its counterpart.*

Keywords: *Bacillus thuringiensis, Compositional Chemical analysis, Endotoxins, MON810: Ajeeb YG®, Safety assessment.*

I. Introduction

The production of GM plants is depending on insertion of new individual gene or genes of interest to the plant. This process causes intended effects which fulfill the original objectives of the genetic modification. But this process also might cause unintended effects which cause differences between GM plants and its conventional counterparts; it could potentially be linked to genetic rearrangement and metabolic perturbations [1]. These changes may include alterations in metabolic pathways resulting in increased levels of endogenous toxins or allergens, or lower levels of essential nutrients, or expression of previously silent genes encoding toxins or allergens [2].

Bt corn "MON810: Ajeeb YG®" was modified by inserting Cry1Ab genes from *Bacillus thuringiensis* in corn; these genes produce delta endotoxins in the whole plant [3, 4]. These endotoxins activate in the alkaline environment of insect's gut; and then the insects die within 24 – 48 hours [5].

With respect to safety of genetically modified foods, there are conflicting opinions, some studies reported that genetically modified foods had potentially toxic properties, which could provoke unintended effects of genetic modification and others reported that it is safe for use [6]. Risk assessment strategy for genetically modified plants applied by comparison of genetically modified foods with their conventional counterparts, assumed that conventional counterparts are a similar foods or feeds to genetic modified ones as mentioned in the regulation (EC) NO.1829 / 2003 [7]. This comparison also denoted the concept of "substantial equivalence" [1]. The genetically engineered foods are considered to be "substantial equivalent" to conventional foods when levels of nutrients, allergens, or naturally occurring toxins are not different and their new allergens or toxins detected [8].

Compositional analysis of GM plants is a key element of comparative safety assessment approach in order to identify similarities and potential differences between the GM plants and its conventional counterparts. This study was designed to assess the safety of Bt corn by comparing the compositional analysis of this variety with non Bt corn "Ajeeb". This analysis includes: Proximate analysis of "moisture, total ash, vitamins, minerals, macro-nutrients and micro-nutrients", anti-nutrients and lipid profile and amino acid profile [1, 9].

II. Materials And Methods

2.1 Sample Preparation

Seeds of Bt corn "MON810: Ajeeb YG®" & non Bt corn "Ajeeb" were carefully cleaned and disqualified from broken seeds. The seeds were milled into a fine powder

2.2 Chemical Compositional Analysis

Compositional analysis was carried out by determination of moisture content, ash, and crude fiber as described in AOAC 2000 [10] methods 930.15, 942.05, and 950.02, respectively. Lipid extracted by modified Folch method [11, 12]Saccharides extracted from dried, lipid free samples according to [13] method 922.02,

925.05. And the total sugars were determined by phenol-sulfuric acid method (AOAC method 44.1.30), as stated in food analysis book [14].

Starch content was determined as described in [15]. Where, the samples were refluxed with concentrated HCl: dH_2O from 3-4 hours at 90°C. After filtration, NaOH 50% and 2-3 drops of phph were added. The mixture was titrated with HCl, and diluted up to 100 ml with dH_2O. Starch content was determined as glucose using phenol-sulfuric acid method and the resulted concentration was multiplied by 0.9.

Protein content of seeds were extracted according to [16]. Samples were treated by 10% TCA/Acetone, then, centrifuged at 16000 rpm for 3 min at 4°C. The pellets were mixed with 80% methanol plus 0.1 M ammonium acetate once and with 80% acetone once and centrifuged as previously described. After drying the pellets at room temperature to remove residual acetone, 0.4 – 0.8 ml of 1:1 phenol (PH 8) : SDS buffer was added for each 0.1 gm starting material. After 5 min, the mixture was centrifuged; then, the upper phenol phase was transferred into a new 2 ml tube, filled with methanol containing 0.1 M ammonium acetate and stored at – 20°C for 10 min. then centrifuged. The supernatant was carefully discarded. The white pellets washed once with 100 % methanol and once with 80 % acetone and dried at each washing step, then mixed and centrifuged. The protein pellets were allowed to air dry and dissolved in SDS buffer. The protein concentration was determined as described in Bradford method [17].

2.3 Mineral Content

Sodium determined by Mohr procedure (AOAC method 960.29), as stated in food analysis book [18]. Magnesium, potassium, and calcium were determined as described by [19]. Phosphorous content determined according to [20].

2.4 Anti-nutritional Factors

Phytic acid was determined according to [21]. And the concentration was determined from standard curve of phytic acid

Tannins was determined according to the modified vanillin – HCl methanol method as described by [22]. Its concentration was determined from the catechin standard curve.

2.5 Fatty Acids Profile

Fatty acids were estimated by GC-MS as described in HP manual [23] where, 1 µl of concentrated extract was injected in a Hewlett-Packard (HP) 5890 gas chromatography that complied with HP-5989 quadrupole mass spectrometer applied with electron ionization mode (EI) generated at 70 eV. The column used was fused-silica (30m long * 0.25mm I.D * 0.25 µm film). The carrier gas was helium with a flow 1.1 ml/min. The column temperature was held for 5 min at 30 °C, and then a rate of 10°C/min up to 245°C. The quadrupole and the electron ionization were maintained at 150°C and 230°C, respectively. The detector temperature was 245°C, and the oven temperature was increased from 70°C up to 220°C with increase rate 2°C/min. The formed methyl esters were identified by comparing its retention time to the retention time of standard methyl esters of fatty acids.

2.6 Amino Acids Profile

Amino acids were analyzed using Amino Acid Analyzer (Dionex ICS – 3000), and a column (AminoPac PA10 analytical and guard column). The defatted sample was hydrolyzed in 1 ml of 6 M HCl and prepared according to [24]. And then, 25 µl of the prepared sample was injected into the column at 30°C and operating backpressure < 3,000 psi. This process was done in the presence of NIST SRM 2389 standard which diluted to 500x with Nleu/azide diluent and eluents: E1 (18.2 megohm water), E2 (250 mM NaOH), and E3 (1 M sodium acetate) with flow rate 0.25 ml/min.

2.7 Statistical Analysis

The statistical analysis was performed using a SPSS 19 program. Data were expressed as mean ± standard error (SE) and statistical significance was assigned at $P \leq 0.05$ level. An independent sample t-test was conducted to compare the compositional chemical of Bt corn & non Bt corn.

III. Results And Discussions

3.1 Chemical Compositional Analysis

The chemical composition of Bt corn "Mon-810: Ajeeb YG®" and its counterpart non-Bt corn "Ajeeb" were determined and results were expressed on the dry weight basis as presented in "Table 1". Moisture content of Bt corn was 13.63% whereas it was 12.06% in non-Bt corn. These values are within the commercial range of moisture content (9.4 – 14.4 %) as published by [25]. Crude fat was 3.23 & 2.96% in Bt & non Bt corn,

respectively. Crude fat was lower than the range of 3.6 – 5.3% as reported by [25], but it was in agreement with the range of 2.6 – 3.3% as listed by [26]. Protein content in Bt corn was 11.6%, this value was higher than that found in Non Bt corn (7.8). As a matter of fact, these values are in agreement with the range of 6 – 12% as published by [26]. Total saccharides content was 6.77% & 6.47% in Bt corn & non Bt corn, respectively. This result is higher than that published by [27] where the total saccharides content in Bt corn was 2.33% & 2.1% for non Bt corn. Starch content ranged from 41.46% for Bt corn to 45.21% for non Bt corn. These values are lower than that reported by [27] being 60.72 & 54.2 % for Bt & non Bt corn, respectively. Crude fiber content was 3.92 % & 3.09 % in Bt and non Bt corn, respectively. The commercial range of crude fiber content was 3.7% in non Bt corn as reported by [25], while the value was 3.4% in Bt corn as listed in [26]. The ash content in non Bt corn was 3.09% and it was 1.67% in Bt corn. The ash content in non Bt corn ranged from 1.1% to 3.9% as reported by [25], whereas it ranged from 1.4% to 1.6% in Bt corn as listed in [26].

3.2 Mineral Content

The mineral content results of Bt corn and its counterpart non Bt as expressed on dry weight basis, is presented in "Table 2". Results indicated that phosphorous exhibited the highest concentration in both of Bt and Non Bt corn, followed by potassium, sodium, calcium and magnesium in Bt corn, and sodium, calcium, potassium and magnesium in non Bt corn.

Magnesium content was 103 mg in non Bt corn and 51 mg in Bt corn. Its concentration in non Bt corn was in agreement with the range of 82 - 1000 mg as reported by [25], but, Bt corn was obviously less than the aforementioned range. Sodium content was 383.3 mg and 230 mg for non Bt and Bt corn, respectively. These values are higher than 0 – 150 mg ranges that were reported by [25]. Calcium content in non Bt and Bt corn was 233.2 & 183.3 mg/100 gm, respectively. This values were higher than 3 - 100 mg ranges that reported by [25] and lower than 390 - 590 mg ranges reported by [28]. Potassium content in non Bt corn was 126.7 mg, but it was 376.7 mg in Bt corn. Potassium content ranged from 320 to 720 mg as reported by [25]. Phosphorous content ranged from 815.64 mg for non Bt corn to 2103.3 mg /100g for Bt corn. These values were higher than the 234 – 750 mg/100g as reported in [25].

3.3 Anti-nutritional Factors

Anti-nutrients content are shown in "Table 3". Tannins content was 1.7 & 1.22 mg in non Bt and Bt corn, respectively. Tannin values in both samples were higher than the range of 0 -0.04 mg as shown by [29] and 0.57 mg as noted in [30]. Phytic acid content was 98 mg in non Bt corn, and it was 63.3 mg in Bt corn. These values are lower than that reported by [29], but, were within the levels 45 - 100 mg as published in [25].

3.4 Amino Acids Profile

Amino acids content in Bt and non Bt corn are shown in "Table 4". A lot of variability has been reported by different researchers in the amino acids content of corn.In non bt corn; arginine, lysine & glycine values were within the expected range of non Bt corn which is 2.9-5.9, 2-3.8 &2.6-4.7, respectively. But valine, threonine, isoleucine, histidine, phenyl alanine, methionine, alanine, serine & cystine values were higher than the expected values of 5.2, 3.9, 4, 2.8, 5.7, 2.1, 9.9, 5.5 & 1.6, respectively. meanwhile, proline, glutamic acid & aspartic acid values were lower than the expected values of 10.3, 19.6 & 7.2, respectively [26]. It was also reported by [25] that valine, isoleucine, histidine, phenyl alanine, methionine, in addition to arginine, lysine &glycine were within the expected range. But, threonine, alanine, serine, & cystine values were higher than the expected range. Whereas, leucine, glutamic acid, & aspartic acid values were lower than the expected range. As a matter of fact, Bt corn values for leucine, proline, & methionine were within the expected range of 7.8- 15.2, 6.6-10.3 & 1-4.6, respectively. But, lysine, histidine, phenyl alanine, isoleucine, & cystine values were higher than the expected range of 3.8, 2.8, 5.7,4, & 1.6, respectively. While, glutamic acid & aspartic acid values were lower than the expected range of 12.4 & 5.8 that was reported in [26]. It was also reported by [25] that isoleucine, leucine, methionine, proline, & cystine values were within the expected range. Whereas, lysine, histidine & phenyl alanine values were higher than 5.5, 3.8, & 6.4, respectively and Glutamic acid and aspartic acid values were lower than 12.5 & 4.8, respectively.

3-5 Fatty Acids Profile

The fatty acids content are shown in "Table 5". As it can be seen, the fatty acids in Bt corn were significantly different from that of non Bt corn, except for myristic, stearic, arachidic & ecosenoic acid. Palmitic acid was 24.41, 20.16 in non Bt & Bt corn, respectively. These values were higher than the range of 7-19 that was reported by [26]. Stearic acid in Bt & non Bt corn was 3.09 & 2.63, respectively. These values were within the expected range of 1-3 for both of Bt & non Bt corn as listed in [26]. Oleic acid in non Bt corn was 1.87 and it was 66.91 in Bt corn. Oleic acid in non Bt corn was quite matching with that reported by [25] but was

significantly lower than that reported by [26] which ranged between (20 – 46). But the value in Bt corn was higher than both ranges. Other fatty acids were not reported in those references.

Table 1: Compositional chemical analysis of non Bt and Bt corn

Parameter	Non Bt	Bt
Moisture %	12.06 ± 0.07 [b]	13.63 ± 0.03 [a]
Crude fat %	2.96 ± 0.03 [b]	3.23 ± 0.07 [a]
Protein %	7.8 ± 0.1 [b]	11.6 ± 0.12 [a]
Total saccharides %	6.47 ± 0.003 [b]	6.77 ± 0.3 [a]
Crude fiber %	3.09 ± 0.09 [b]	3.92 ± 0.03 [a]
Starch content %	45.21 ± 2.09 [a]	41.46 ± 5 [a]
Ash %	3.09 ± 0.09 [a]	1.66 ± 0.02 [b]

Each value is mean ± SE
* The same letter in the same row is not significant different at (P≤ 0.05)

Table 2: Mineral content of Non Bt & Bt corn mg/100g

Element	Non Bt	Bt
Mg	103 ± 10 [a]	51 ± 3 [b]
Na	383.3 ± 0.00 [a]	230 ± 76.7 [a]
Ca	233.3 ± 10.4 [a]	183.3 ± 10.2 [b]
K	126.7 ± 14.5 [b]	376.7 ± 29.6 [a]
P	815.64 ± 73.23 [b]	2103.3 ± 510.8 [a]

Each value is mean ± SE
* The same letter in the same row is not significant different at (P≤ 0.05)

Table 3: Anti-nutrients content of Non Bt & Bt corn (mg/100g)

Parameter	Non Bt	Bt
Phytic acid	98 ± 4.41[a]	63.3 ± 4.41 [b]
Tannins	1.7 ± 0.06 [a]	1.22 ± 0.16 [a]

Each value is mean ± SE
* The same letter in the same row is not significant different at (P≤ 0.05)

Table 4: Amino Acid composition of Non Bt and Bt corn

Amino acid	Non Bt	Bt
Essential Amino Acids		
Arginine	3.1	ND
Lysine	3.7[b]	23.1[a]
Threonine	7.4	ND
Valine	5.3	ND
Isoleucine	6.4 [a]	5.8 [b]
Leucine	3.7 [b]	9.3 [a]
Methionine	3.1[a]	3.1[a]
Histidine	3.8 [b]	7.3[a]
Phenyl alanine	6.3[b]	15.7[a]
Non - Essential Amino Acids		
Alanine	29.4	ND
Glycine	3.2	ND
Serine	22.7	ND
Proline	4.3[b]	9.2[a]
Glutamic acid	0.3[b]	7.8[a]
Aspartic acid	1.7[a]	1.3[b]
Cystine	3.4[a]	2.4[b]

Values are expressed as a percent of total protein
* The same letter in the same row is not significant different at (P≤ 0.05)
ND: Not Detected

Table 5: Fatty Acid Composition of Non Bt & Bt corn		
Fatty acid	**Non Bt**	**Bt**
Myristic acid (C_{14}:0)	0.62^a	0.7^a
Palmitic acid (C_{16}:0)	24.41^a	20.16^b
Palmitoleic acid (C_{16}:1)	0.43	ND
Stearic acid (C_{18}:0)	2.63^a	3.09^a
13, Octadecenoic acid	62.86	ND
Olein, 2-mono	1.14^b	1.32^a
Oleic acid (C_{18}:1)	1.87^b	66.91^a
Arachidic acid (C_{20}:0)	0.54^a	0.6^a
11, Eicosenoic acid (C_{20}:1)	0.44^a	0.55^a
Behenic acid (C_{22}:0)	0.19^b	0.3^a
Ligoceric acid (C_{24}:0)	0.23^b	0.33^a
Values are expressed in (mg/100 g)		
* The same letter in the same row is not significant different at ($P \leq 0.05$)		
ND: Not Detected		

IV. Conclusion

Obviously, the genetic modification of Bt corn showed significant differences from the conventional counterpart, where, the total protein, crude fat, crude fiber & total saccharides showed significant increase in Bt corn as compared to non Bt corn. Whereas ,the starch content showed significant decreased compared to non Bt. The Mineral content were also affected, where calcium & sodium were significantly decreased in Bt corn, while phosphorous increased dramatically in Bt corn. All fatty acids were detected with various values in Bt corn compared to non Bt corn except for Palmitoleic acid & 13- octadecenoic acids were not detected in Bt corn and most probably lost. In respect to amino acids, some essential and non essential amino acids were lost in Bt corn. Thus, it may be conclude that the genetic modification process caused several alternation in the chemical composition in corn that may be toxic to the human food and the animals feed. Accordingly, further long term feeding studies are required to assess the actual safety of Bt corn.

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