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Composition of Transgenic Soybean Seeds with Higher γ-Linolenic Acid Content Is Equivalent to That of Conventional Control

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ABSTRACT: γ-Linolenic acid (GLA) has been used as a general nutraceutical for pharmacologic applications, particularly in the treatment of skin conditions such as eczema. Four transgenic soybean lines that produce GLA at high yields (4.21% of total fatty acids, up to 1002-fold) were generated through the stable insertion of the Delta-6-fatty acid desaturase gene isolated from *Borago officinalis* into the genome of a conventional soybean cultivar. As part of the safety assessment of genetically engineered crops, the transgenic soybean seeds were compared with their parental soybean seeds (nontransgenic) by applying the principle of substantial equivalence. Compositional analyses were conducted by measuring the fatty acids, proximate analysis (moisture, crude protein, crude fat, carbohydrates, TDF, and ash contents), amino acids, lectins, and trypsin inhibitor activity. The present results showed that the specific transgenic cultivar studied was similar to the conventional control.

KEYWORDS: *γ-linolenic acid, transgenic soybean, composition, substantial equivalence*

INTRODUCTION

γ-Linolenic acid [GLA, C18:3 Δ(6,9,12)] is an intermediate in the metabolic pathway of essential fatty acids from linoleic acid (LA) to arachidonic acid in human and animal diets. GLA is also the direct precursor of dihomo-c-linolenic acid, which is a precursor of anti-inflammatory 1-series eicosanoids. GLA deficiency may occur when D6-desaturation activity decreases due to aging, stress, diabetes, eczema, and some infections, or when GLA is catabolized due to oxidation or more rapid cell division (e.g., in cancer or inflammation).[1,2] Dietary supplementation with GLA is effective in treating a number of such conditions (e.g., atopic eczema, diabetic neuropathy, viral infections, and cancer).[3−5]

GLA is a rare fatty acid, only available from relatively few plant species [e.g., borage (*Borago officinalis* L.), evening primrose (*Oenothera biennis* L.), and black currants (*Ribes nigrum* L.)], from which cost-effective production is difficult.[6−8] One potential strategy to reduce production cost would be to generate them in major oilseed crops. Soybean (*Glycine max* L. Merrill) is one of the most important oil-producing crops, and soybean seeds have no or very low content of GLA. However, these seeds have rich LA content [C18:2 Δ(9,12)], which could be converted to GLA by Delta-6-fatty acid desaturase (Δ^6-*fad*). Our research group has isolated the Δ^6-*fad* gene from *B. officinalis*, transformed it successfully into soybean, and obtained several transgenic soybean lines. After five rounds of testing cultivation, ten transgenic soybean lines were found to have normal agronomic characteristics compared with the control of the same background while maintaining their higher GLA content.[9,10] These transgenic soybeans are expected to become excellent potential sources of GLA for nutraceutical and pharmaceutical applications.

With the introduction of the exogenous Δ^6-*fad* gene, the biological metabolic balance of soybean can be disrupted, which can cause a series of accidental reactions that will result in both physiologic and ecological problems. Thus, transgenic soybeans must be checked thoroughly for safety to both the environment and the consumer when they are used for food. Accordingly, many regulations have been implemented for the safety assessment of these genetically modified products, of which the scientific concept of substantial equivalence has been accepted widely. The concept suggests that if no meaningful difference from the conventional counterpart is found, then the transgenic crop is as safe and nutritious as its traditional counterpart, which is generally accepted as safe based on human food use history.[11] Composition studies are considered an essential part of the safety assessment of new crop varieties, including those related to transgenic soybean seeds. Therefore, the present research was designed to evaluate the composition of transgenic soybean seeds compared with that of its conventional counterpart, and to determine if significant compositional changes were induced by the insertion of the Δ^6-*fad* gene into the soybean genome or by the heterologous expression of Δ^6-*fad*, which is based on the Organization for Economic Cooperation and Development consensus document guidelines.[12,13]

MATERIALS AND METHODS

Soybean Materials and the Experiment. Ten transgenic soybean lines, TS88, TS89, TS810, TS813, TS815, TS816, TS817, TS819, TS824, TS825, and the conventional control Pudou-8808 (S8) were commonly cultivated in the cultivation pots in a greenhouse in South China Agricultural University in Guangzhou, China. A plot of each cultivar (one transgenic soybean line or its control S8) was composed of five cultivation pots of soybeans. The experiment (the soybean cultivation pot arrangement) was performed in the completely randomized block design with three replicates. The size of soybean cultivation pot was 30 cm in diameter and 23 cm in height. The transgenic soybean lines were fifth generation of self-crossing

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progenies from T0 generation of soybean transformants, which were produced through the *Agrobacterium*-mediated transformation of conventional soybean cultivar Pudou-8808 (S8) with plasmid vector pLIN61. The plasmid vector contains the Δ^6-*fad* gene isolated from *Borago officinalis* and the *bar* gene isolated from *Streptomyces hygroscopicus* as a selectable marker. Two lines (TS824 and TS825) were integrated with the Δ^6-*fad* gene and could produce high yields of GLA only. Others were integrated with both the Δ^6-*fad* gene and *bar* gene, and they could supply GLA and confer tolerance to glufosinate.

Compositional Analysis. *Assay of Fatty Acids.* Up to 20 g of transgenic soybean and nontransgenic soybean seeds, respectively, were ground into powder using a high-speed tissue masher.[14] Their fatty acid profiles were calculated through gas chromatography and were performed by the China National Analytical Center, Guangzhou (www.fenxi.com.cn).

Proximate Analysis. The standard methods of the Association of Official Analytical Chemists were adopted to determine the levels of proximate compositions, including moisture, ash, carbohydrate, crude fat, total dietary fiber (TDF), and crude protein.[15] Moisture content and ash were determined in an oven at 105 and 550 °C, respectively, until a constant weight was attained. The Kjeldahl method[16] was used to determine the seed total nitrogen and protein content, which was calculated using a nitrogen conversion factor of 6.25. Fat content was determined using the Soxhlet extraction method, whereas TDF was determined using the enzymatic−gravimetric method. The carbohydrate content was calculated by subtracting the contents of crude protein, fiber, fat, and ash from 100% dry matter.

Analysis of Amino Acid. Protein hydrolysis was performed before ninhydrin derivatization to analyze total amino acid composition, with the exception of Cys, Met, and Trp. Seed powder (10 mg) was hydrolyzed with 1 mL of 6 M HCl at 110 °C for 22 h under a nitrogen atmosphere. The hydrolysate was dried under reduced pressure, dissolved in 0.02 M HCl, and was subjected to analysis using the ninhydrin method using a UV detector and a Hitachi L-8800 automatic amino acid analyzer (Hitachi High-Technologies, Tokyo, Japan) equipped with an ion-exchange column (4.6 mm × 60 mm). The temperature of the separation column was at 57 °C. The buffer flow rate was 0.40 mL/min, and the pressure of the buffer pump was 12 Pa. The ninhydrin flow rate was 0.30 mL/min, and the pressure of the ninhydrin pump was 1.1 kPa. The cystine and cysteine in the samples were oxidized to cysteic acid, and methionine was oxidized to methionine sulfone through performic acid treatment for 16 h at approximately 0 °C. After acid hydrolysis, the sample was separated on an anion exchange column and detected with a ninhydrin reaction, as previously described.[17]

Lectin Analysis. Lectins are univalent or polyvalent proteins of nonimmune origin that bind reversibly and noncovalently to specific sugars on apposing cells, thus precipitating polysaccharides, glycoproteins, and glycolipids that bear specific sugars. Lectins were determined by measuring the agglutinating properties of soybean sample extract on rabbit red blood cells according to modified procedures from the literature.[18,19,21]

Trypsin Inhibitor Activity. The protease inhibitor assay was carried out by a slight modification of the method.[20] Defatted soybean meal (0.02 g) was suspended in 1 mL of 0.01 M NaOH, which was stirred magnetically for 3 h. After this period, the mixture was left for 30 min without stirring, and then 0.5 mL of the supernatant was mixed with 0.5 mL of 0.01 M NaOH in an Eppendorf centrifuge tube. This solution was centrifuged for 5 min at 14000*g*. After centrifugation, 0.1 mL of the alkaline extract was mixed with 1.6 mL of 0.05 M Tris-HCl, at pH 8.2, containing 0.02 M $CaCl_2$, 0.1 mL of trypsin (Sigma, type I) solution, and 0.1 mL of *N*-α-benzoyl-L-arginine *p*-nitroanilide solution. The mixture was incubated for 45 min at 37 °C, and then 0.2 mL of 30% acetic acid solution was added. The absorbance at 410 nm was measured, and the amount of trypsin inhibited was calculated from a calibration curve using soybean trypsin inhibitor (Sigma, type I-S).

Statistical Analysis. The statistical data analyses were performed using the SPSS 15.0 package. All analytical determinations were conducted in triplicate, and data are presented as mean ± standard deviation. Statistical analysis was carried out using an ANOVA. If a significant *F*-test was noted, then the means were separated using Duncan's multiple-range test.[22] Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fatty Acid Profiles of Transgenic Soybean Lines and the Control. The levels of fatty acids changed considerably between the control and ten transgenic soybean lines, including GLA, LA, and oleic acid (Table 1). Analysis of the gas chromatograph showed a novel peak (8.204 min, Figure 1B), corresponding to the standard GLA, and the highest percentage of GLA to total fatty acids in the transgenic seeds (TS815) was 4.21% (in 1002-fold increments compared with CK (0.0042%, 8.138 min, Figure 1A)), which indicates that the Δ^6-*fad* gene has been successfully expressed at high levels in the transgenic soybean. The GLA of TS817 was the lowest among the transgenic soybeans, but it was 417 times higher compared with the control S8-CK. The results also showed that the LA and oleic acid content varied significantly between the ten transgenic soybeans and the control, especially between TS824 and S8-CK, and significant differences were observed among the different transgenic soybeans, in which the contents of LA varied from 5.54% to 8.67% and oleic acid contents varied from 3.81% to 7.57% (Table 1). These changes may be caused by the introduction of the exogenous Δ^6-*fad* gene.

Table 1. Fatty Acid Composition of the Transgenic Soybean and Control Seeds (%)[a]

soybean lines	γ-linolenic acid (GLA)	linoleic acid (LA)	oleic acid	fold increments of GLA
S8-CK	0.0042	7.41	3.32	
TS88	3.01	7.82	5.41	716
TS89	3.02	5.93	4.83	719
TS810	2.16	8.67	4.98	514
TS813	3.83	7.92	5.22	911
TS815	4.21	7.36	4.14	1002
TS816	2.83	8.02	3.81	673
TS817	1.98	7.54	6.38	471
TS819	4.19	8.25	5.67	997
TS824	3.06	5.54	7.57	728
TS825	2.48	7.91	5.48	590

[a]% denotes the fatty acid grams per 100 g of dry weight of soybean seeds.

GLA is an intermediate in the metabolic pathway of essential fatty acids from oleic acid (OA) to linoleic acid (LA) to γ-linolenic acid (GLA) to arachidonic acid or to dihomo-c-linolenic acid in human and animal diets. These research results showed that the transgenic soybean lines produced GLA one hundred times higher than the control line but their precursor OA and LA contents were similar even though they varied in a broad range. This indicated that the Δ^6-*fad* gene transfer significantly increased the GLA production and accumulation but did not have significant effects on other compositions around the fatty acid pathway.

GLA, one of the essential and nutritional polyunsaturated fatty acids in human and animal diets, plays an important role in hormone regulation and fatty acid metabolism.[24] In our previous study, the Δ^6-*fad* gene was successfully transformed into soybean through *Agrobacterium*-mediated transformation and has been expressed at high levels in a transgenic soybean. In the present study, the GLA content of T_5 generations of mark-free transgenic soybeans that produce GLA was detected. In contrast with the control, the percentage of GLA to total

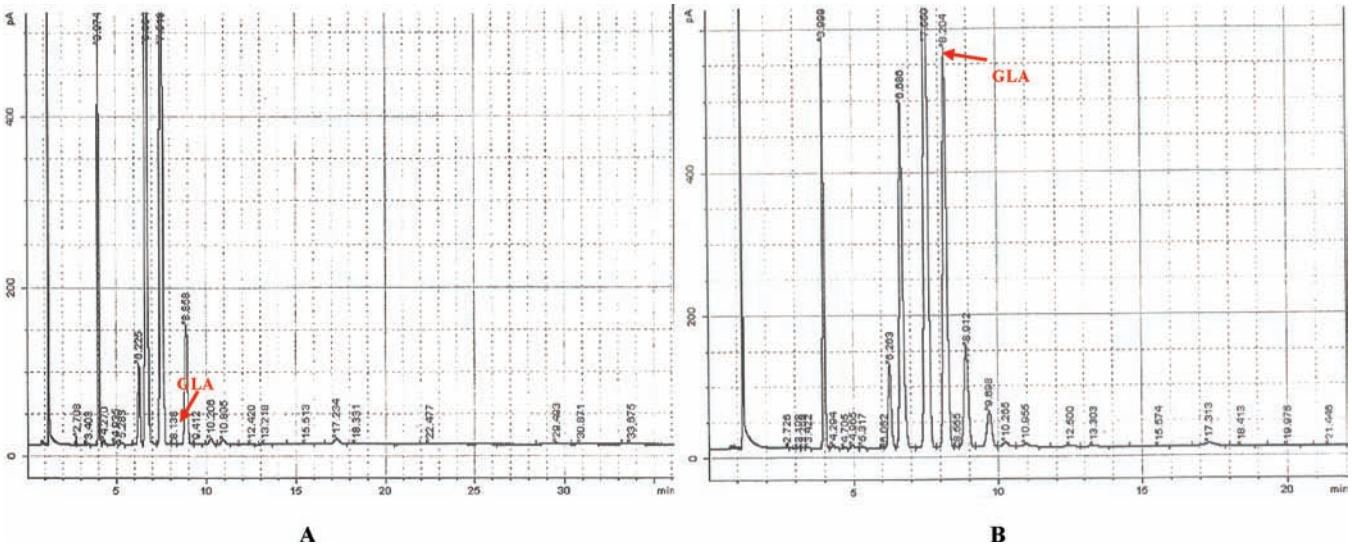

Figure 1. Gas chromatograms of the fatty acids obtained from the control S8-CK (A) and the transgenic soybean TS815 (B). (A) The peak retention time and area of GLA in the control are 8.138 min and 0.0042% of total fatty acids, respectively. (B) The peak retention time and area of GLA in the transgenic soybean TS815 are 8.204 min and 4.2% of total fatty acids, respectively (not all figures attached).

Table 2. Proximate Composition of the Transgenic Soybean and Control Seeds (Mean ± SD; n = 3)[a]

component[b]	S8-CK	TS813	TS815	TS819	TS824	lit. range[c]
moisture	8.11 ± 0.06	8.08 ± 0.08	8.12 ± 0.06	8.21 ± 0.05	8.23 ± 0.07	7−11
crude protein	37.13 ± 0.14	38.65 ± 0.09	38.27 ± 0.13	36.94 ± 0.18	37.62 ± 0.15	36.9−46.4
crude fat	20.45 ± 0.09 b	22.36 ± 0.06 a	20.23 ± 0.11 b	22.08 ± 0.08 a	21.01 ± 0.14 ab	13.2−22.5
carbohydrate	24.02 ± 0.11	22.58 ± 0.18	23.07 ± 0.15	22.42 ± 0.17	21.91 ± 0.23	30.9−34.0
TDF	5.84 ± 0.30	5.87 ± 0.18	5.82 ± 0.06	5.93 ± 0.20	5.87 ± 0.35	4.7−6.48
ash	4.45 ± 0.09	4.46 ± 0.43	4.49 ± 0.30	4.42 ± 0.38	4.46 ± 0.16	4.61−5.37

[a]In each row, different lowercase letters indicate significant difference ($p < 0.05$). No lowercase letter indicates no significant difference. [b]Grams per 100 g of dry weight (moisture based on fresh weight). [c]From refs 23 and 24.

fatty acids in a transgenic soybean was 4.21% in TS815 (in 1002-fold increments, Figure 1B) and 4.19% in TS819 (in 997-fold increments). These transgenic soybeans produced higher GLA and herbicide resistance, which would provide a larger substrate pool for GLA production and feeds.

Proximate Compositions. From the above results, we chose four transgenic soybean lines (TS813, TS815, TS819, and TS824) with high GLA yield to check the changes of components in these transgenic soybean lines. Table 2 shows the proximate analyses (moisture, crude protein, crude fat, carbohydrates, total dietary fiber, and ash content) of the five soybean seeds. Moisture was calculated based on fresh weight. No statistically significant differences were observed in moisture content, not only between the transgenic soybean seeds and the control but also among the different transgenic soybean seeds. Content of other chemicals is presented based on dry weight. The ANOVA showed that crude fat values varied between two transgenic soybean seeds (TS813 and TS819) and the control ($p < 0.05$), but that there were no significant differences between two other transgenic soybean seeds (TS815 and TS824) and the control. The presence of significant difference in crude fat may be caused by the successful transformation and expression of the Δ^6-*fad* gene in the transgenic soybeans, but the analyzed mean content of each was within the range reported previously for soybeans.[23,24] No significant difference was observed in terms of crude protein, carbohydrate, TDF, and ash contents between the transgenic soybeans and the control. These results implied that the proximate compositions of the transgenic soybean line TS813, TS815, TS819, and TS824 were substantially equivalent to the control S8-CK except for the intended composition of crude fat in the transgenic soybean line TS813 and TS819.

Amino Acid Contents. Table 3 presents the amino acid contents of the five soybean cultivar seeds. Up to 18 amino acids were detected in the five cultivars, and all amino acids were identical in the profile.[23,24] Statistical analysis revealed that TS813 was significantly different from the control in terms of glutamic acid and proline content, but those of TS815, TS819, and TS824 were not significantly different from the control. TS815 was significantly different from the other transgenic soybean seeds and the control in histidine content. Despite these differences, the values were within the published literature ranges[23,24] and there were no significant differences in the levels of any of the other 15 amino acids measured in the transgenic soybean seeds and the control. All soybean seeds contained high percentages of essential amino acids (EAA) and total amino acids (TAA), and there were no significant differences in TAA and EAA content among the soybean seeds. These results demonstrated that the amino acid contents of the transgenic soybean cultivars were substantially equivalent to the control soybean cultivar.

Antinutrient Composition. Lectins and trypsin inhibitors are two important antinutritional factors present in soybean seeds. Table 4 shows the results of the antinutritional factor analysis. The concentrations of both the lectins and trypsin inhibitors were generally low and did not exceed the range reported previously, which might not affect the nutritional potential of soybean seeds. Statistical analysis revealed that there was

Table 3. Amino Acid Composition of the Transgenic Soybean and Control Seeds (Mean ± SD; $n = 3$)[a]

component[b]	S8-CK	TS813	TS815	TS819	TS824	lit. range[c]
alanine	1.53 ± 0.03	1.56 ± 0.04	1.62 ± 0.03	1.65 ± 0.02	1.53 ± 0.03	1.49−1.87
arginine	2.82 ± 0.04	2.93 ± 0.03	2.82 ± 0.05	2.90 ± 0.01	2.79 ± 0.05	2.45−3.49
asparagine	3.89 ± 0.04	4.19 ± 0.05	4.16 ± 0.04	3.98 ± 0.02	3.85 ± 0.01	3.87−4.98
cysteine	0.61 ± 0.01	0.62 ± 0.02	0.61 ± 0.01	0.63 ± 0.02	0.63 ± 0.01	0.56−0.66
glutamic acid	6.80 ± 0.05 a	6.59 ± 0.03 b	7.10 ± 0.04 a	6.97 ± 0.02 a	7.09 ± 0.06 a	6.10−8.72
glycine	1.97 ± 0.02	1.90 ± 0.01	1.99 ± 0.03	2.01 ± 0.02	1.95 ± 0.02	1.88−2.02
histidine	1.20 ± 0.01 a	1.20 ± 0.02 a	0.95 ± 0.03 b	1.05 ± 0.02 a	1.01 ± 0.01 a	0.89−1.08
isoleucine	1.58 ± 0.02	1.55 ± 0.03	1.52 ± 0.02	1.60 ± 0.03	1.56 ± 0.03	1.46−2.12
leucine	2.83 ± 0.05	2.79 ± 0.03	2.86 ± 0.06	2.93 ± 0.03	2.88 ± 0.06	2.71−3.20
lysine	2.46 ± 0.02	2.43 ± 0.03	2.54 ± 0.02	2.50 ± 0.03	2.48 ± 0.02	2.71−3.20
methionine	0.51 ± 0.02	0.55 ± 0.03	0.53 ± 0.01	0.50 ± 0.01	0.53 ± 0.01	0.49−0.66
phenylalanine	1.83 ± 0.02	1.88 ± 0.01	1.90 ± 0.02	1.81 ± 0.01	1.85 ± 0.03	1.70−2.08
proline	1.92 ± 0.01 a	1.89 ± 0.02 b	1.95 ± 0.01 a	1.96 ± 0.02 a	2.01 ± 0.03 a	1.88−2.61
serine	2.13 ± 0.01	1.99 ± 0.02	2.01 ± 0.01	2.11 ± 0.02	2.20 ± 0.01	1.81−2.32
threonine	1.51 ± 0.01	1.49 ± 0.02	1.53 ± 0.02	1.60 ± 0.01	1.51 ± 0.02	1.33−1.79
tryptophan	0.58 ± 0.01	0.61 ± 0.01	0.59 ± 0.02	0.57 ± 0.01	0.59 ± 0.01	0.49−0.66
tyrosine	1.40 ± 0.02	1.45 ± 0.02	1.35 ± 0.03	1.43 ± 0.02	1.38 ± 0.01	1.12−1.62
valine	1.68 ± 0.01	1.71 ± 0.01	1.65 ± 0.01	1.70 ± 0.01	1.63 ± 0.02	1.52−2.24
TAA	37.21	37.33	37.68	37.72	37.37	
EAA	13.97	14.06	14.04	14.3	13.97	

[a]In each row, different lowercase letters indicate significant difference ($p < 0.05$). No lowercase letter indicates no significant difference. [b]Grams per 100 g of dry weight. [c]From refs 23 and 24

Table 4. Summary of Lectin and Trypsin Inhibitor of the Transgenic Soybean and Control Seeds (Mean ± SD; $n = 3$)[a]

	S8-CK	TS813	TS815	TS819	TS824	lit. range[d]
lectin (HU/mg)[b]	6.11 ± 0.02	6.08 ± 0.01	6.12 ± 0.01	6.17 ± 0.03	6.13 ± 0.02	5.6−6.6
trypsin inhibitor (TIU/mg)[c]	43.25 ± 1.14	43.05 ± 1.89	42.97 ± 1.13	42.94 ± 1.78	42.92 ± 0.95	42.9−45.0

[a]In each row, different lowercase letters indicate significant difference ($p < 0.05$). No lowercase letter indicates no significant difference. [b]Fresh weight basis. HU = hemagglutinating units. HU/mg denotes lectin units per milligram of fresh weight of soybean seeds. [c]TIU = trypsin inhibitor units. [d]From refs 23 and 24.

no significant difference in antinutritional factors between transgenic soybean seeds and the control seed. These results exhibited that the antinutrient compositions of the transgenic soybean lines were substantially equivalent to the control line.

In conclusion, if these transgenic soybeans are to be used as food or feeds, a safety assessment program for a genetically engineered soybean is necessary to develop a good understanding of transgenic soybean uses in animals and humans. Safety assessment studies conducted on genetically modified soybean were based on the application of the principle of substantial equivalence, which has been adopted by leading international food and regulatory bodies, including the World Health Organization,[26] the United Nations Food and Agricultural Organization,[27] the Organization for Economic Cooperation and Development,[28] and the International Life Sciences Institute.[29−32] According to this principle, transgenic soybean seeds were compared with their control, and the results of compositional analysis show no significant difference in the most detected components between the transgenic soybean seeds and the control S8-CK. These detected components, excluding some fatty acids (GLA, LA, and oleic acid), exhibited similar nutritional profiles with the comparator S8-CK, and were within the normal ranges reported for soybean seeds. In addition, examination of the data indicated that no analyses showed a consistent trend of difference between the transgenic soybean seeds and the control S8-CK. Therefore, based on the data presented in the present study and the published literatures, the transgenic soybean TS813, TS815, and TS819 are substantially equivalent to the control S8-CK except in terms of the GLA content and the tolerance to glufosinate, and the transgenic soybean marker-free line TS824 is substantially equivalent to the control S8-CK with respect to these important constituents.

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Notes

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REFERENCES

(1) Horrobin, D. F. Gamma linolenic acid: An intermediate in essential fatty acid metabolism with potential as an ethical pharmaceutical and as a food. *Rev. Contemp. Pharmacother.* **1990**, *1*, 1−45.

(2) Simon, J. A.; Chen, Y. H.; Bent, S. The relation of α-linolenic acid to the risk of prostate cancer: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* **2009**, *89*, 1597−1601.

(3) Gill, I.; Valivety, R. Polyunsaturated fatty acids, part 1: Occurrence, biological activities and applications. *Trends Biotechnol.* **1997**, *15* (10), 401−409.

(4) Hounsom, L.; Corder, R.; Patel, J. Oxidative stress participates in the breakdown of neuronal phenotype in experimental diabetic neuropathy. *Diabetologia* **2001**, *44*, 424−428.
(5) Menendez, J. A.; Delmar, B. Effects of gamma-linolenic acid and oleic acid on paclitaxel cytotoxicity in human breast cancer cells. *Eur. J. Cancer* **2001**, *37*, 402−413.
(6) Syed Rahmatullah, M. S. K.; Shukla, V. K. S.; Mukherjee, K. D. γ-Linolenic Acid Concentrates from Borage and Evening Primrose Oil Fatty Acids *via* Lipase-Catalyzed Esterification. *J. Am. Oil Chem. Soc.* **1994**, *71* (6), 563−567.
(7) Belch, J. J.; Alexander, H. Evening primrose oil and borage oil in rheumatologic conditions. *Am. J. Clin. Nutr.* **2000**, *71*, 352−356.
(8) Simoncikova, P.; Wein, S.; Gasperikova, D. Comparison of the extrapancreatic action of γ-linolenic acid and n-3 PUFAs in the high fat diet-induced insulin resistance. *Endocr. Regul.* **2002**, *36*, 143−149.
(9) Zhang, X. C.; Lin, J. F.; Guo, L. Q. Cloning of Δ^6-desaturase gene and construction of its co-transformation expression vector. *Chin. J. Trop. Crops* **2004**, *25* (4), 63−67.
(10) Zhang, X. C.; Lin, J. F.; Peng, M.; Guo, L. Q. Characterization of Marker-free Transgenic Soybeans with Δ∼6-fatty Acid Desaturase Gene Expressing γ-linolenic Acid. *J. Trop. Subtrop. Bot.* **2006**, *14* (6), 460−466.
(11) Ramessar, K.; Peremarti, A.; S Gómez-Galera, S. Biosafety and risk assessment framework for selectable marker genes in transgenic crop plants: a case of the science not supporting the politics. *Transgenic Res.* **2007**, *16* (3), 261−280.
(12) Organization of European Cooperation and Development (OECD). *Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients*; Series on the Safety of Novel Foods and Feeds, No. 2; Organization of European Cooperation and Development: Paris, France, 2001.
(13) Organization of European Cooperation and Development (OECD). *Consensus Document on Compositional Considerations for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites*; Series on the Safety of Novel Foods and Feeds, No. 6; Organization of European Cooperation and Development: Paris, France, 2002.
(14) Rui, Y. K.; Wang, W.; Zhang, F. S.; Lu, Y. A new kind of fatty acid emerged in transgenic cotton seed. *Riv. Ital. Sostanze Grasse* **2007**, *84*, 39−42.
(15) AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2002.
(16) Baethgen, W. E.; Alley, M. M. Optimizing soil and fertilizer nitrogen use by intensively managed winter wheat. I. Crop nitrogen uptake. *Agron. J.* **1989**, *81*, 116−120.
(17) Association of Official Analytical Chemists. *Official Methods of Analysis*, 15th ed.; AOAC: Arlington, VA, 1990.
(18) Liener, I. E. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. *Arch. Biochem. Biophys.* **1955**, *54*, 223−231.
(19) Klurfeld, D. M.; Kritchevski, D. Isolation and quantitation of lectins from vegetable oils. *Lipids* **1987**, *22*, 667−668.
(20) Hamerstrand, G. E.; Black, L. T.; Glover, J. D. Trypsin inhibitors in soy products: Modification of the standard analytical procedure. *Cereal Chem.* **1981**, *58*, 42−45.
(21) Martin, S.; Jost, W.; Hans-Curt, F. Application of fluorescently labelled lectins for the visualization and biochemical characterization of polysaccharides in biofilms of *Pseudomonas aeruginosa*. *J. Microbiol. Methods* **2002**, *50*, 237−248.
(22) Wahua, T. A. T. *Applied Statistics for Scientific Studies*; African Link Press: Aba, 1999.
(23) Taylor, N. B.; Fuchs, L. R.; MacDonald, J.; Ahmed, R.; Shariff, A. R.; Padgette, S. R. Compositional Analysis of Glyphosate-Tolerant Soybeans Treated with Glyphosate. *J. Agric. Food Chem.* **1999**, *47* (10), 4469−4473.
(24) Harrigan, G. G.; Ridley, W. P.; Riordan, S. G.; Nemeth, M. A.; Sorbet, R.; Trujillo, W. A.; Breeze, M. L.; Schneider, R. W. Chemical Composition of Glyphosate-Tolerant Soybean 40−3-2 Grown in Europe Remains Equivalent with That of Conventional Soybean (*Glycine max* L.). *J. Agric. Food Chem.* **2007**, *55* (15), 6160−6168.
(25) Wang, J.; Guo, L. Q.; Lin, J. F. Composition of Transgenic *Volvariella volvacea* Tolerant to Cold Stress Is Equivalent to That of Conventional Control. *J. Agric. Food Chem.* **2009**, *57*, 2392−2396.
(26) World Health Organisation (WHO). *Report of a Joint FAO/WHO Consultation*; World Health Organisation: Geneva, Switzerland, 1991.
(27) World Health Organisation (WHO). *Report of a WHO Workshop*; World Health Organisation, Food Safety Unit: Geneva, Switzerland, 1995.
(28) Food and Agriculture Organisation of the United Nations (FAO). Report of a Joint FAO/WHO Consultation, Rome, Italy, 1996.
(29) OECD, Organisation for Economic Cooperation and Development, Concepts and Principles, Paris, France, 1993.
(30) OECD, Organisation for Economic Cooperation and Development, Food Safety and Evaluation, Paris, Franc, 1996.
(31) OECD, Organisation for Economic Cooperation and Development, Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods, Paris, France,1997.
(32) ILSI Europe Novel Foods Task Force. *Food Chem. Toxicol.* **1997**, *34*, 931−940.