ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN
2019 International Nuclear Atlantic Conference - INAC 2019
Santos, SP, Brazil, October 21-25, 2019

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3

GLYCINE MAX OIL PHYSICAL-CHEMICAL QUALITY OBTAINED OF IRRADIATED SEEDS

José Gilmar Franco[1], Regildo Márcio Gonçalves da Silva[2], Rodrigo Yukio Suguimoto[2] Suely Salumita Haddad Franco[3], Anna Lucia Casañas Haasis Villavicencio[1], Valter Arthur[3,1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
zegilmar60@gmail.com

[2]Laboratório de Fitoterápicos, Departamento de Ciências Biológicas, Faculdade de Ciências e Letras de Assis, São Paulo,

[3,1]Centro de Energia Nuclear na Agricultura (CENA / USP)
Departamento de Radiobiologia e Meio Ambiente
Universidade de São Paulo
13416-000 Piracicaba - SP. Brasil.
arthur@cena.usp.br

ABSTRACT

Ionizing radiation applied to agriculture has mainly benefited food production, as it reduces natural losses caused by physiological processes, as well as eliminating or reducing microorganisms, parasites and pests. In addition, this technique also allows the production of mutants with characteristics of greater productivity, precocity, smaller size, greater resistance to diseases and pests. These mutants are used to obtain new varieties of species of agronomic interest. The objective of this study was to evaluate the physic-chemical quality of the oil extracted from seeds of *G. max* (soybean) obtained from irradiated seeds with different doses of gamma radiation (0, 25, 50, 75 and 100 Gy of a Cobalt-60 source, type Gammacell-220 installed in CENA-USP). The physic-chemical analyzes included: AOCS Cd-3d-63, AOCS Cd-3-25 and AOCS Cd-1-25, Acids Index (I.A), Saponification Index (IS), Iodide Index (II), respectively. All analytical determinations were performed at least in triplicates. The values found for I.A., for I.S. and for I.I., did not differ from the oil sample obtained from soybean seeds from control subjects (without irradiation) regardless of the concentration and dose of radiation applied to the seeds. The oil analyzed did not show physical-chemical variation in comparison with the oil obtained from non-irradiated seeds, thus suggesting the absence of modifications in these parameters after the genetic improvement induced by the radiation.

1. INTRODUCTION

In consideration of the characteristics of ionizing radiation, many areas are benefited by its application, from Medicine, Industry (mainly Pharmaceuticals), Agriculture and even the War Industry. Among its applications stand out: radioactive tracers; radio sterilization of medical equipment and instruments; radiodiagnosis (tomography and mammography); radiopharmaceuticals; radiotherapy among others [1]. Among these areas, agriculture has benefited from the use of techniques that use radiation, where food irradiation reduces natural losses caused by physiological processes, as well as eliminating or reducing microorganisms, parasites and pests without causing any damage to food. In addition to improving the shelf life of food, this technique also allows the production of mutants with characteristics of greater productivity, precocity, smaller size, greater resistance to diseases, pests and lodging. These mutants are used to obtain new varieties of species of agronomic interest [2].

Within this context, radiation becomes an important tool for mutation induction, a technique that seeks to increase genetic variability in plant breeding. One of the methods used is the irradiation of plant seeds for aggregation of agronomic values such as resistance to some diseases and tolerance to toxic metals in the soil, such as aluminum [3]. Several studies have been carried out to study the potential of controlled seed irradiation as an effective tool for large-scale genetic improvement [4].

Despite the radiation technique in agriculture, both for food irradiation as a tool for genetic improvement, to be safe from a physical and biological point of view, other analyzes are necessary to corroborate with the use of this technique. Among these analyzes is the *Alium cepa* L. Test that allows for the phytotoxic, cytotoxic and genotoxic evaluation of chemical substances, waste and industrial waste, water and sediments of rivers and effluents, as well as monitoring substances present in the environment and contaminants and radiation [5,6,7,8,9] which is based on the results obtained in the literature.

With regard to ionizing radiation, it can be considered a vector of high mutagenic potential, since it can act directly on organic molecules by modifying the DNA structure. Example of the use of the *Allium cepa* test was the genotoxic evaluation on soils of inhabited and contaminated regions by Cs137 after the Chernobyl accident in Ukraine, resulting in a dose-dependent relationship between the increase in the number of chromosomal aberrations and the fall of the mitotic rate insofar as the soil was more contaminated [10]. Another example of the application of the test was the analysis of the impact of the high level of natural radiation on the soil of the inhabited region of Ramsar in Iran [11]. In relation to irradiated foods the test was carried out to evaluate the genotoxic potential of *Allium sativum* L. (garlic) irradiated with Co60, the study showed that, despite the incentive to irradiate the food, these can present high genotoxic potential when submitted to specific doses , which may lead to cytotoxicity and genotoxicity when ingested by other organisms [12].

Given the importance of the study of the genotoxic potential of irradiation as an agricultural tool and the applicability of the A. cepa test for the detection of genotoxic events, studies and investigations are necessary to elucidate biological and environmental risks in the use of this technique.

The present work aims to evaluate the genotoxic potential and the oil quality of G. max seeds (soybean) produced by individuals from irradiated seeds. The Allium cepa test was used to

evaluate mitotic process disorders, chromosomal aberrations and micronucleus frequency, and the oil quality evaluation was performed according to international standards for the production of biofuel.

2. MATERIAL AND METHODS

2.1. Radiated Seeds of *G. max*.

The seeds of G. max were obtained from the Center for Nuclear Energy in Agriculture (CENA) of the University of São Paulo, Piracicaba-SP, Laboratory of Radiobiology and Environment. The seeds were irradiated at doses of 25, 50, 75 and 100 Gy. For the irradiation of the seeds, a source of Cobalt 60 (Co60), type Gammacell-220 was used. Seeds without irradiation were also used as controls. The seed irradiation process was carried out at CENA-USP. The seeds were sown and planted in an experimental farm belonging to the Center for Nuclear Energy in Agriculture (CENA) of the University of São Paulo in the municipality of Palmital-SP, with cultivation and own management for soybeans and with support and technical sup

2.2. Extraction and characterization of the oil of *G. max*.

The soybean seeds were macerated to facilitate the penetration of the solvent and consequently to optimize the extraction of the oil. Subsequently, moisture was determined for each sample after drying in a forced air oven with an average temperature of 100ºC, and the weight of each sample was measured every 12 hours for 48 hours, if necessary. After obtaining constant weight for each sample, about 20 grams of it were wrapped in filter paper and the whole was transferred to 250 mesh stainless steel capsules and placed in a Soxhlet fat and lipid extractor to remove the oil. Three different solvents were used: ethanol, methanol and hexane and two mixtures of ethanol / methanol and methanol / hexane in proportions of 1: 1 and 3: 1. The Soxhlet extraction set was initially heated for 2, 4, 6, 8 and 10 hours to determine the optimal extraction time. After extraction the solid material contained in the capsule was transferred to oven to constant weight and the material contained in the flask was transferred to rotary evaporator until complete removal of the solvent. The quantities of pie and oil taken from the seed were determined by gravimetry.

2.3. Physico-chemical characterization of oils

The physico-chemical characterization of the oils was performed using standard methods (AOCS, 1990 and 1993), as described below: (a) Iodine Index (II) by AOCS method Cd-1-25, similar to ASTM D5554-95 , which allows the determination of the degree of unsaturation of an oil by the percentage of iodine absorbed by the sample; (b) AOCS Method Cd-3d-63, similar to ASTM D664-06a, which is associated with the number of milligrams of potassium hydroxide required to neutralize free acids in one gram of sample; (c) Saponification Index (IS) by AOCS method Cd-3-25, similar to ASTM D5558-95, which may be defined as the amount of potassium hydroxide required to saponify a defined amount of sample. The SI was used to calculate the mean molar mass (MM) of the forage turnip oil sample, which was later used to calculate the number of regents and quantification of the products of the transesterification reactions.

2.4. Determination of toxicity in meristematic cells of *Allium cepa* root

To verify the genotoxic activity of the water resulting from cyanotoxin tests on *Allium cepa* L. root (onion) seed cells were previously germinated in Petri dishes containing germination paper moistened with distilled water, when the roots of the seedlings reached 1 cm in length were used to assemble a completely randomized design experiment (DIC). Each plot consisted of a Petri dish containing 25 onion seedlings, in four replicates per treatment.

The onion seedlings were exposed to the water resulting from the tests for a period of 48 hours, after which time they were placed in a Petri dish containing distilled water until they reached 5 cm in length (recovery period) and, finally, the collection of the roots. The whole experiment was carried out under conditions of relative humidity, temperature and luminosity artificially controlled in greenhouses of germination type BOD. After collection, the roots were fixed in Carnoy (absolute ethyl alcohol and glacial acetic acid, 3: 1) for 8 hours at room temperature, after which time Carnoy's solution was replaced with a freshly prepared solution and the roots were stored in (± 4ºC) until the preparation and analysis of the slides.

2.5. Preparation and Analysis of Blades of *Allium cepa*

The roots were hydrolyzed in 1N hydrochloric acid (HCl) at 60 ° C for 8 minutes, and then stained in Schiff's Reagent for 2 hours in the dark. The roots were placed on slides and a drop of 2% acetic Carmin was added over the meristem and the covers were covered by coverslips. Then they were crushed and fixed in the flame of the lamp. The analyzes were performed under an optical microscope (increase of 100x with the aid of immersion oil). Five roots were used per plot, where 5000 cells / treatment were observed. The cytotoxic effects of the extracts were determined by mitotic index analysis (total number of cells in division / total number of cells analyzed x 100) and cell death index (total number of cells at death / total number of cells analyzed x 100). Chromosomal aberrations were also determined (aberrant anaphase and telophase), and their frequencies were used to determine the index of chromosomal changes (total number of cells altered / total number of cells analyzed x 100). Micronucleate cell frequency determination was used to determine the mutagenic effects by analyzing the mutagenicity index (number of cells with micronucleus and breaks / total number of cells analyzed x 100). For the statistical analysis of the A. strain strain the results were submitted to the non-parametric tests: Kruskal-Wallis and Mann-Whitney (significance level of 5% and 1% analysis) according to [11].

3. RESULTS AND DISCUSSION

Table 1 shows the values found for I.A., I.S., I.R and I.I. of the oils obtained from seeds of G. max (soybean) produced by individuals from irradiated seeds. Where it is possible to verify that the values of the indices did not differ from the oil sample obtained from soybean seeds from control subjects (without irradiation) independent of the concentration and dosage of exposed radiation.

Table 1. Results of physico-chemical analyzes of soybean oil obtained from seeds of *G. max* (soybean) produced by individuals from irradiated seeds.

Samples	Parameter evaluated	Results
0 Gy	Acid Value	2.012± 0.1 mg NaOH/g oil
	Saponification Index	130± 6.9 mg KOH/g oil
	Index of Refraction	14.20±0.001
	Iodine content	52.30±1.76 mg I_2/100g oil
25 Gy	Acid Value	2.22± 0.1 mg NaOH/g oil
	Saponification Index	147± 3.9 mg KOH/g oil
	Index of Refraction	13.70±0.001
	Iodine content	47.18±5.16 mg I_2/100g oil
50 Gy	Acid Value	2.069± 0.1 mg NaOH/g oil
	Saponification Index	134± 4.7 mg KOH/g oil
	Index of Refraction	11.80±0.001
	Iodine content	50.11±3.23 mg I_2/100 oil
75 Gy	Acid Value	2.014± 0.1 mg NaOH/g oil
	Saponification Index	148± 3.9 mg KOH/g oil
	Index of Refraction	12.5±0.001
	Iodine content	54.93±2.16 mg I_2/100g oil

Gy - dosage of radiation imposed on the seeds that originated the individuals producing seeds used in the study to extract the evaluated oil.

Table 2 shows the results of the mitotic indices of the meristematic root cells of *A. cepa* strain exposed to 10% solutions of Oil that received different doses of radiation (0,25 and 100 Gy) diluted in Tween 80. According to the results it was possible to verify that there was no significant difference in relation to the mitotic index between the treatments Tween, Oil 0, Oil 25 and Oil 100 Gy and that there was significant difference in comparison to the positive control group (MMS) and negative.

Table 2. Mitotic index of *Allium cepa* root meristematic cells treated with Tween extracts, Oil 0, 25 Gy Oil, 100 Gy Oil, Negative Control (CN) Distilled Water and Positive Control Methylmethanesulfonate (MMS).

Extracts	Cell division				Índex Mitotic
	Prophase	Metaphase	Anaphase	Telophase	
CN	89.20±46.10	9.40±6.65	6.20±4.43	8.60±5.32	11.52±5.52a
Tween	47.75±30.63	7.25±6.80	7.00±7.39	9.75±12.76	5.39±3.25b
Oil 0	174.50±53.03	34.00±7.07	10.50±0.70	35.0±5.65	5.91±2.30b
Óil 25 Gy	67.00±42.63	7.75±3.86	6.75±2.06	6.75±4.85	7.05±4.14b
Óil 100 Gy	30.71±23.03	2.57±1.13	4.57±2.93	5.71±4.608	5.71±2.37b
MMS	64.40±13.50	13.00±7.30	4.60±3.57	15.40±6.02	9.74±2.50c

Results expressed by mean ± standard deviation. Equal letters in the same column did not differ significantly Kruskal-Wallis and Mann-Whitney (significance level of analysis of 5% and 1%).

Table 3 shows the values of death rates, chromosomal changes and mutagenicity of *A. cepa* strain root meristematic cells exposed to 10% solutions of Oil that received different doses of

radiation (0, 25 and 100 Gy) diluted in Tween 80 According to the results, it was possible to verify that there was no significant difference in relation to the Cell Death Index and the Index of chromosomal alterations between the treatments Tween, Oil 0, Oil 25 Gy and the negative control, however there was a significant difference in comparison to the treatment 100 Gy Oil and the positive control group (MMS). For the mutagenicity index, there was no significant difference between the treatments and the negative control, and there was a significant difference in comparison with the positive control.

Table 3. Mitotic index, death index, index of chromosomal changes and Mutagenicity index of *Allium cepa* meristematic root cells treated with Tween extracts, Oil 0, 25 Gy Oil, 100 Gy Oil, Negative Control (CN) Distilled Water and Control Positive Methylmethanesulfonate (MMS).

Extracts	Death Index	Chromosomal change index	Mutagenicity index
CN	0.26±0.19a	0.26±0.10a	0.0a
Tween	0.27±0.03a	0.20±0.07a	0.04±0.001a
Oil 0	0.25±0.08a	0.21±0.08a	0.04±0.001a
Óil 25Gy	0.06±0.01a	0.21±0.03a	0.04±0.009a
Oil 100Gy	0.07±0.02b	0.41±0.07b	0.03±0.003a
MMS	3.20±1.17c	1.20±0.59c	1.22±0.077b

Results expressed by mean ± standard deviation. Equal letters in the same column did not differ significantly Kruskal-Wallis and Mann-Whitney (significance level of analysis of 5% and 1%).

Table 4 shows the results of determination of the micronucleus frequency and chromosomal aberrations of the meristematic root cells of *A. cepa* strain exposed to 10% solutions of Oil that received different doses of radiation (0, 25 and 100 Gy) diluted in Tween 80. In the analysis of the determination of micronucleated cells, it was observed that there was no statistical difference in the micronucleus frequency between the treatments 100 Gy and the negative control and that there was a statistical difference in the frequency of micronuclei between treatments Tween, Oil 0,25 Gy in comparison to the positive control. For the determination of aberrant metaphase no significant difference was observed between the Tween, 0 Gy Oil and negative control treatments, and they differed statistically in comparison with both the positive control and the 25 and 100 Gy treatments. However, the aberrant anaphase analysis showed no significant difference between the treatments 25, 100 Gy and the negative control, and they differed statistically in comparison with both the positive control and the treatments Tween and Oil 0 Gy. For the aberrant telophase analysis the Tween, 25 Gy Oil and 100 Gy Oil treatments did not differ from each other and compared to the negative control, differing only from the positive control and the 0 Gy Oil treatment.

Table 4. Chromosomal aberrations (AC), Micronuclei (MN) and Chromosomal losses of *Allium cepa* meristematic root cells treated with the extracts Tween, Oil 0,25 Gy Oil, 100 Gy Oil, Negative Control (CN) Distilled water and Positive Control Methyl methanesulfonate (MMS).

Extracts	Micronucleus	Aberrant Metaphase	Aberrant Anaphase	Aberrant Telophase
CN	0.0a	1.20±0.17a	0.600±1.342a	0.2±0.04a
Tween	0.25±0.05b	1.00±2.00a	1.500±1.291b	0.0a
Oil 0 Gy	0.50±0.07b	1.00±1.41a	3.000±4.243b	1.00±0.40b
Oil 25 Gy	0.25±0.05b	0.25±0.50b	0.0a	0.0a
Oil 100 Gy	0.0a	0,28±0.08b	0.0a	0.0a
MMS	5.40±2.40c	7.40±0.30c	6.600±5.595c	1.00±0.22b

Results expressed by mean ± standard deviation. Equal letters in the same column did not differ significantly Kruskal-Wallis and Mann-Whitney (significance level of analysis of 5% and 1%).

4. CONCLUSION

With the present work, it can be concluded that the oil obtained from seeds of individuals from irradiated *G. max* seeds does not present differences for I.A., I.S., I.R and I.I. in comparison to the oil obtained from seeds of individuals originating from non-irradiated seeds. At determination of the genotoxic potential by the test method with *A.cepa* strain soybean oil samples showed no genotoxic and cytotoxic activity.

REFERENCES

1. E. M. Cardoso. Aplicações da energia nuclear, *Apostila Educativa de Aplicações da Energia Nuclear*, p. 03-09, 1999.

2. K. Karunakaran, I. S. Kiss, K. Mikaelsen. Mutagenic effectiveness and efficiency of gamma rays, fast neutrons and ethyl methane sulphonate in rice. *Technical Reports Series*, v. 131, p. 91-96, 1971.

3. C. E. O. Camargo, A. W. P. F. Filho, A. T. Neto, A. P. Junior, J. P. Castro, J. C. Felicio, , J. C. Mistro, M. V. Salomon. Avaliação de linhagens de trigo originárias de hibridação com e sem irradiação gama. *Bragantia*, v. 64, p. 61-74, 2005.

4. J. W. Preiss, S. Klein. Depth Controlled Deuteron Irradiation of *Lactuca sativa* Seeds. *Plant Physiology*, v. 33, p. 326-339, 1958.

5. G. Fiskesjo. *Allium* test on copper in drinking water. *Vatten,* v. 37, p. 232-240, 1981.

6. J. Rank, A. G. Jensen, B. Skov, L. H. Pedersen, K. Jensen. Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrowmicronucleus test, *Salmonella* mutagenicity test, and *Allium cepa* anaphase-telophase test. *Mutation Research,* v. 300, p. 29-36, 1993.

7. L. K. S. Chauhan, P. N. Saxena, V. Sundararaman, S. K. Gupta. Diuron-induced cytological and ultrastructural alterations in the root meristem cells of *Allium cepa*, *Pest. Biochem. Physiol.*, v. 62, 152-163, 1998.

8. M. D. Bagatini, A. C. F. Da Silva, S. B. Tedesco. Uso do sistema teste de *Allium cepa* como bioindicador de genotoxicidade de infusões de plantas medicinais. *Revista Brasileira de Farmacognosia*, v. 17, n.3, 2007.

9. M. D. Leme, M. A. Marin-Morales *Allium cepa* test in environmental monitoring: A review on its application, *Mutation Research,* v. 682, p. 71-81, 2009.

10. O. Kovalchuk, I. Kovalchuk, A. Arkhipov, P. Telyuk, B. Hohn, L. Kovalchuk. The *Allium cepa* chromosome aberration test reliability measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident. *Mutation Research***,** v.415, p. 47-57, 1998.

11. M. Saghirzadeh, M. R. Gharaati, S. Mohammadi, M. Ghiassi-Nejad. Evaluation of DNA damage in the root cells of *Allium cepa* seeds growing in soil of high background radiation areas of Ramsar – Iran. *Journal of Environmental Radioactivity*, v. 99, p. 1698-1702, 2008.

12. T. A. Campo, G. J. S. Junior, D. Santiago, L. P. Silva, R. M. G. Da Silva. Avaliação do potencial genotóxico de *Allium sativum L.* irradiado por meio do teste de micronúcleo em células meristemáticas de raiz de *Allium cepa. 60ª Reunião Anual da SBPC*, 2008.