

Full Length Research Paper

Analysis chromatography the *Ocimum basilicum*, *Origanum vulgare*, *Cymbopogon citratus* and *Thymus vulgaris* after gamma irradiation

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The ionizing radiation is a safe and efficient method to eliminate the bacterial of foods and in the disinfestations of fruits, vegetables among other products. However, the importance in determining the correct doses efficient and analyzing the effect of the radiation in the chemistry of the product, nutritional value and in the quality organoleptic if they make necessary. The chromatography analyzes the chemical effect that the treatment with ionizing radiation in foods can cause. The objective of this work was analysis Chromatography the *Ocimumbasilicum*, *Origanumvulgare*, *Cymbopogoncitratus* and *Thymus vulgaris* after gamma irradiation being used the radiation gamma of Cobalt-60. The experiment was developed in the Laboratory of Pharmacology of the InstitutoBiológico/SP during the month of Janeiro of 2007 and the irradiations accomplished at the Institute of Nuclear and Energy Research - IPEN/SP, being used an experimental irradiator of Cobalt-60, model Gammacell 220 at 3.32 kGy/h. The dried and powdered aerial parts (40g) of *Ocimumbasilicum*, *Origanumvulgare*, *Cymbopogoncirtatus* and *Thymus vulgaris* were submitted to extraction with hexane and ethanol (3 times) at room temperature. Solvents were filtered and evaporated under vacuum to yield hexanic (HEX) and ethanolic (EtOH) extracts. The extractions procedures were repeated with the aerial parts of the same plants irradiated with 5 and 10 kGy. The HEX and EtOH extracts were analyzed by thin layer chromatography over TLC aluminium sheets silica gel F254 (Merck) eluted with mixtures of hexane: ethyl acetate (HEX: ACOEt) and chloroform: methanol (CHCl₃:MeOH) in different polarities. The best systems were HEX: ACOEt (8:2) for hexanic extracts and HEX: ACOEt (3:7) for ethanolic extracts. The sheets were analyzed by UV (254 nm) and iodine vapours. These analyses did not show significant chemical differences between irradiated extracts and the control (no irradiated extracts).

Keywords: Control food, Disinfestations in food, Medicinal Plants, Chromatography, Gamma Irradiation.

INTRODUCTION

It enters the modern methods of analysis in foods, the chromatography occupies a place of prominence due to its easiness in effecting the separation, identification and

quantification of the chemical species, for same itself or in set with others instrumental techniques of analysis, as, for example, the espectrofometria or the spectrometry of masses (Collins, 1995).

The chromatography is a separation method where the components separate and are distributed in two phases, one that are stationary while to another one it is put into motion in a direction defined through a porous

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way, dragged by one dissolvent in movement (Braithwaite and Smith, 1996).

The technique of the chromatography has been used during centuries to separate to materials extracts of plants mainly dehydrated. Russian chemistry and Botanical Michael Tswett (1872-1919) was the first one in 1906 to use the term chromatography, the name drift of the words Greeks "chrom" (color) and "graphe" (to write) (Braithwaite and Smith, 1996). Although the process does not depend on the color, except facilitating the identification of the separate components.

The chemical analysis of complex mixtures is one of the spread out and excellent branches more of Analytical Chemistry. A representative sample of this analyzed material is collected according to procedure definite of the chemical, statistical point of view and of the sector interested in the analysis. This definition is important to guarantee the representation of the sample collected in relation to the origin material and the use of the results that will be gotten (Aquino Neto and Souza Nunes, 2003).

Second Aquino Neto and Souza Nunes (2003) one show can be solid, liquid, gaseous or a combination of these physical states. In the end of analytical boarding he must be subdivided in three or four discrete sets of constituent molecules: the analitos of interest, interferences, solvent matrix and or diluente, being defined as: Analitos- Substances or species that if it desires to characterize or to determine quantitatively; Interferences- Substances that directly affect the result of the analysis of the analitos; Matrix - Substances that constitute the sample with exception of the analitos. It is common that it contains polar substances and of high molecular weight. In conceptual terms, it would have to also contain the interferences. It is considered, however, in general way, only those constituent that harm in not specific way the result of the analysis and Solvent - Fluid added to the system with intention to dissolve the analitos being assisted in its separation of the remain of the sample, or mobilization in certain stages of the necessary procedures its characterization.

A current operation, as initial stage in the analysis process is the isolation of the analitos of its interferences and the matrix, folloied of the concentration of these analitos. This allows to greater trustworthiness in the analysis and characterization of the analitos in miniature amounts. In general, this is made with greater or lesser success, for extraction of the analitos of the way where they are contained, for solvent intermediary of an appropriate one. When the sample will be diluted (gas, liquid or supercritical fluid) or already will be in half fluid (if the analitos of interest will have been extracted of the original material for a solvent), has been given bigger emphasis to the use of "solvent" in the solid state, through the technique of Extraction for Solid Phase. It presents many advantages in relation to the extractions

with solvent liquids or supercritical fluids (Aquino Neto and Souza Nunes, 2003).

MATERIALS AND METHODS

The experiment was developed in the Laboratório de Farmacologia of the Instituto Biológico/SP during the month of Janeiro of 2007 and the irradiations accomplished at the Instituto de Pesquisas Energéticas e Nucleares - IPEN/SP, being used an experimental irradiator of Cobalt-60, model Gammacell 220 at 3.32 kGy/h. The dried and powdered aerial parts (40g) of *Ocimum basilicum*, *Origanum vulgare*, *Cymbopogon nectratus* and *Thymus vulgaris* were submitted to extraction with hexane and ethanol (3 times) at room temperature. Solvents were filtered and evaporated under vacuum to yield hexanic (HEX) and ethanolic (EtOH) extracts. The extractions procedures were repeated with the aerial parts of the same plants irradiated with 5 and 10 kGy. The HEX and EtOH extracts were analyzed by thin layer chromatography over TLC aluminium sheets silica gel F254 (Merck) eluted with mixtures of hexane: ethyl acetate (HEX: ACOEt) and chloroform: methanol (CHCl₃: MeOH) in different polarities. The best systems were HEX: ACOEt (8:2) for hexanic extracts and HEX: ACOEt (3: 7) for ethanolic extracts. The sheets were by UV (254 nm) and iodine vapours.

RESULTS AND DISCUSSION

It was found that gamma radiation significantly increased up to 70% the efficiency of ethanolic extract of *L. esculentum* in killing *S. zeamais* adults at dose of 5 kGy. The doses used yielded mortality efficiency values ranging between 10 and 30% for the aqueous extracts of *L. esculentum*. The gamma radiation in doses used, promoted efficiency of aqueous extract of *L. esculentum*, between 12 and 20% (Table 1). The irradiated and no irradiated aqueous extract of *N. pectinata* yielded zero efficiency. The doses of 7.5 e 10 kGy cancel the efficiency of hexanic and ethanolic extracts, and in the dose of 5 kGy both extracts showed 36% of efficiency (Table 1). The gamma radiation showed adverse effect on the aqueous extract of *R. graveolens*, decreasing its efficiency of 20% to 12%, 6% and 2% in doses of 5; 7.5 and 10 kGy, respectively. In addition, a reduction in the efficiency of the hexanic extract was also observed for doses higher than 5 kGy (Table 1). Significant effects were not observed of gamma radiation on aqueous, hexanic and ethanolic extracts of *F. elastica* (Table 1).

The Chromatographic Analysis of Thin Layer was to evaluate this research did not show chemical differences in the extracts analyzed, e. g. Figures 1, 2, 3 and 4.

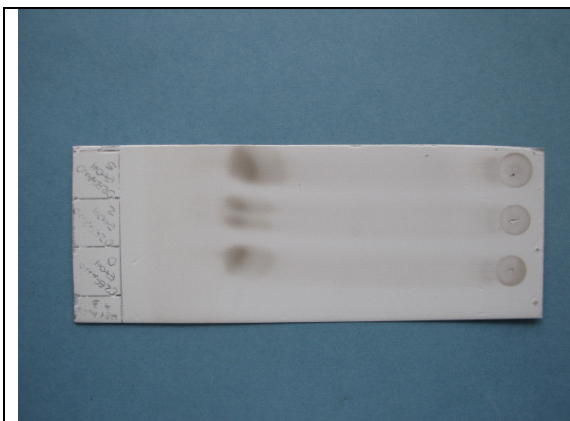


Figure 1. *Cymbopogon citratus* S radiated in the doses of 0, 2, 5 kGy. Chromatographic analysis (CCD) ruda hexânico extract (HEX) in the ratio of HEX 8:2 AcOEt.

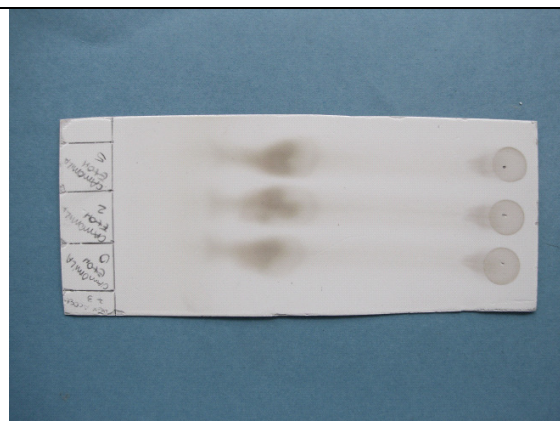


Figure 2. *Cymbopogon citratus* S. radiated in the doses of 0, 2, 5 kGy. Chromatographic analysis (CCD) ruda etanólico extract (EtOH) in the ratio of HEX 3:7 AcOEt.

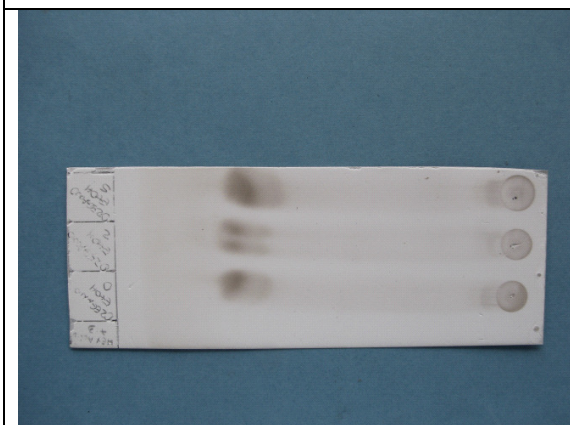


Figure 3. *Origanum vulgare* L radiated in the doses of 0, 2, 5 kGy. Chromatographic analysis (CCD) ruda hexânico extract (HEX) in the ratio of HEX 8:2 AcOEt.

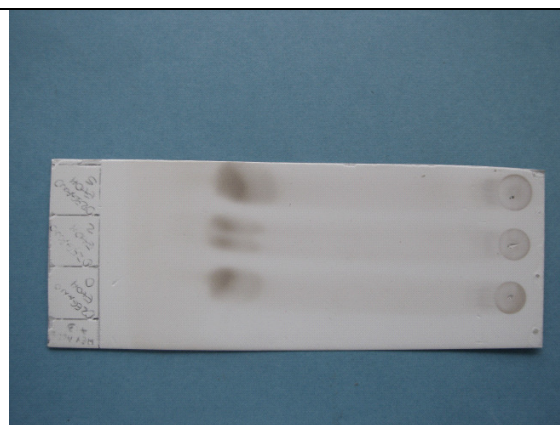


Figure 4. *Origanum vulgare* L. radiated in the doses of 0, 2, 5 kGy. Chromatographic analysis (CCD) ruda etanólico extract (EtOH) in the ratio of HEX 3:7 AcOEt.

CONCLUSION

The Chromatographic Analysis of Thin Layer was to evaluate did not show chemical differences in the extracts analysed.

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REFERENCES

Alasbimjournal: Revista de Medicina Nuclear. Disponível em: <<http://www.alasbimjournal.cl/revistas/14/posters/06/03aliment os.html>>. Acesso em: 05 de julho de 2004.

- Al-kahtani HA, Abu-Tarboush HM, Al-dryhim YN, Ahmed MA, Bajaber AS, Adam EE, El-Mojaddidi MA (1998). Irradiation of dates: insect disinfestation, microbial and chemical assessments, and use of thermoluminescence technique. *Radiation Physics and Chemistry*, v. 53, p. 181-187.
- Braithwaite A, Smith FJ (1996). *Chromatographic Methods*. New York, N. Y.: Blackie Academic and Professional
- Cetinkaya N, Ozyardimeci, Denli E, IC E (2006). Radiation processing as a post-harvest quarantine control for raising dried figs and dried apricots. *Radiation Physics and Chemistry*, v. 75, p. 424-431
- Collins CH (1995). Aspectos Históricos da Cromatografia. In: Collins, C. H., Braga, G. L., Bonato, P. S. *Introdução a Métodos Cromatográficos*. 6. ed. São Paulo: Editora da Universidade Estadual de Campinas- UNICAMP, cap.1, Aspectos Históricos da Cromatografia. p.13-19.
- Curzio OA, Croci CA, Domarco RE, Spoto MHF, Blumer L, Walder JMM (1997). Avaliação da qualidade do alho irradiado na Argentina e armazenado no Brasil. In: ENCONTRO DE APLICAÇÕES NUCLEARES, 4., 18-22 agosto, Minas Gerais. Resumos. Minas Gerais.
- De Penna EW (1999). Métodos Sensoriales Y Sus Aplicaciones. In: Almeida, T. C. A.; Hough, G.; Damásio, M. H.; Da Silva, M. A. A. P. *Avances En Análisis Sensorial*. São Paulo. Programa Iberoamericano de ciência y tecnologia para el desarrollo, p. 13-22.

- Farkas J (1985). Radiations processing of dry food ingredients – a review. *Radiat. Phys. Chem*, v. 25, nos. 1-3, p. 271-280
- Josephson S, do E, Thomas MH, Calhoun WK (1979). Aspectos nutritivos da irradiação de alimentos: Uma Visão Geral. *J. Alimento Proc. Preserv*, v. 2, p. 299-313
- Kilcast D (1994). Effect of irradiation on vitamins. *Food Irradiation and the Chemist*, v.49.p. 157-164,
- Koseki PM, Villavicencio ALCH, Brito MS, Nahme LC, Sebastião KI, Rela PR, Almeida-muradian LB, Mancini-filho J, Freitas PCD (2002). Effects of irradiation in medicinal and eatable herbs. *Radiation Physics and chemistry*, v. 63, p. 681-684
- Lopes JLC (1995). Os Adsorventes. In: Collins, C. H., Braga, G. L., Bonato, P. S. *Introdução a Métodos Cromatográficos*. 6. ed. São Paulo: Editora da Universidade Estadual de Campinas- UNICAMP, p.47-57.
- Madruça MS, Aldrigue ML (2002). Análises Químicas, Físico-Químicas e Físicas de Alimentos. In: Aldrigue, M. L. Editora Universitária/Ideia. *Aspectos da Ciência e Tecnologia de Alimentos*. João Pessoa, v.1. p.11-57.
- Matsuda AH (2002). Aplicação da técnica de irradiação gama para preservação de própolis, - Dissertação (Mestrado) – Instituto de Pesquisas Energéticas e Nucleares, São Paulo
- OMS-Organização Mundial Da saúde (1989). *La irradiación de los alimentos una técnica para conservar y preservar la inocuidade de los alimentos*, Ginebra
- Oms-Organização Mundial Da Saúde (1995). *Inocuidad e Idoneidad nutricional de los alimentos irradiados*. Ginebra
- Owczarczyk HB, Migdal W, Kedzia B (2000). The pharmacological activity of medical herbs after microbiological decontamination by irradiation. *Radiation Physics and Chemistry*. Poland, v. 57, p.331-335
- Ribeiro E (1992). *Plantas medicinais e complementos bioterápicos*. Portugal: Europa-América
- Soemartaputra MH, Haryadi RS, Rahayu A, Kardha S, Purwanto ZI, Chosdu R (1991). Radiation Disinfections of Tobacco Bales and Coffee Beans. In: *International Symposium on Food Irradiation*, March 8, 1985, Vienna. *Proceedings...* Vienna: IAEA, p.170.
- WHO (1994). "High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy", *Technical Reports Series 890*, Geneva