

EVALUATION OF FUNGAL BURDEN OF MEDICINAL PLANTS SUBMITTED TO GAMMA RADIATION PROCESS AFTER 30 DAYS

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ABSTRACT

Forty samples of medicinal plants (*Peumus boldus*, *Camellia sinensis*, *Maytenus ilicifolia* and *Cassia angustifolia*), purchased from pharmacies and street market in the five cities of São Paulo State, were irradiated using a ⁶⁰Co gamma ray source (Gammacell) with a dose of 10.0 kGy, delivered at dose rate of 3.0 kGy/h. Non-irradiated samples were used as controls of fungal isolates. For fungal counts and identification in medicinal plants a serial dilutions from 10⁻¹ to 10⁻⁶ of the samples were seeded in duplicates and plated using the method in Dichloran 18% Glycerol Agar (DG 18) and were counted after five days at 25°C. Mycological analysis of control samples revealed the presence of genera *Aspergillus* and *Penicillium*, which are known as toxigenic fungi. The process of gamma radiation was effective in reducing the number of colony forming units (cfu/g) in all irradiated samples of medicinal plants after 30 days, using the dose of 10.0 kGy and kept of veiled conditions.

1. INTRODUCTION

The past decade has seen a significant increase in the use of herbal medicine. Despite lots of studies on the mycoflora in agricultural products, only a few are concerned with spices and herbal medicines that are more and more common in our daily diet and play an important role in the economy [1]. Some investigators recorded the presence of moulds in medicinal plants, drugs and herbs that are widely consumed as home remedies and raw materials used for the pharmaceutical industries [2, 3].

In some kinds of the tested medicinal plants, the percentage of contamination in packed samples was higher than those non-packed and this may be due to other factors, such as, rise of humidity inside the pack and also unsuitable methods of keeping and storing of the pack [2].

To reduce fungal contamination in medicinal plants, an average dose of 5.0 kGy was enough to reduce the count to acceptable levels, as observed in a study carried out by Aziz et al.[4]. Soriani et al. [5] reported that gamma irradiation can be considered effective to improve the microbial quality of ginkgo and guarana and after irradiation up to 17.8 kGy the content of the main active principles was not modified.

2. MATERIALS AND METHODS

2.1. Fungal Isolation and Enumeration

The samples of *Peumus boldus* (Boldo), *Camellia sinensis* (Chá verde), *Maytenus ilicifolia* (Espinheira santa) and *Cassia angustifolia* (Sene) were portioned in 10g and were homogenized for 30 min., in separated bottles containing 90 ml sterile distilled water. For fungal counts and identification, 0.1 ml of the dilutions, in a serial dilution from 10^{-1} to 10^{-6} of the samples were seeded in duplicates and plated using the surface method in Dichloran 18% glycerol agar (DG 18). A total fungal concentration was counted after five days at 25 °C.

3.2. Water activity

The water activity (A_w) of the samples was determined in AQUALAB CX-2 equipment from Decagon Devices.

3.3. Gamma Irradiation

The medicinal plants samples were irradiated in polyethylene bags, each containing 10 g of samples, using a ^{60}Co gamma ray source (Gammacell 200) located at Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN), in São Paulo city. The samples were exposed to dose of 10.0 kGy. The gamma ray source gave a dose rate of 3.0 kGy/h.

3. RESULTS AND DISCUSSION

In this present study the results revealed high levels of fungal burden (90%) in non-irradiated samples and indicated that 60% of control samples were above the limit established by WHO [6], for total counting of moulds in colonies forming units per gram (10^3 cfu/g). Romagnoli et al. [1] collected twenty-seven aromatic herbs from markets, shops and bonded warehouse in Italy and subjected to a microbiological investigation to research the presence of moulds. The same author compared the results with the Official Pharmacopoeia of the Italian Republic (IX edition), that determine the values for moulds in herbal infusions or decoctions to 10^4 UFC/g and demonstrated that 56% of samples results contaminated from moulds, according to our study. Soriani et al. [5] revealed high levels of total fungi in non-irradiated samples of ginkgo and guarana and the fungi count was about 10^3 and 10^6 cfu/g, respectively, as found in our results (Table 1).

The isolated species of fungi in control samples belong to the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Syncephalastrum*, *Fusarium*, *Cephalosporium*, *Mucor*, *Absidia* and *Scopulariopsis*. The frequency of fungi genera in Boldo was: *Aspergillus* (50.2%), *Penicillium* (0.62%), *Cladosporium* (49.11%), *Absidia* (0.07%) and *Mucor* (0.04%).

In Sene it was observed the frequency of *Aspergillus* (70%), *Penicillium* (28.5%), *Mucor* (0.7%), *Fusarium* (0.5%), non-sporulated fungi (0.15%) and *Scopulariopsis* (0.15%). The samples of Espinheira santa showed the following frequencies: *Aspergillus* (67.7%), *Penicillium* (5.0%), *Cladosporium*, (2.23%), non-sporulated fungi (13.0%), *Mucor* (1.6%), *Syncephalastrum* (0.16%), *Fusarium* (0.31%) and *Cephalosporium* (10.0%). The Chá verde samples showed *Aspergillus* (63.3%), *Penicillium* (27.7%) and *Syncephalastrum* (9.0%).

The genus *Aspergillus* was the most dominant recovered on medicinal plants samples and it is considered a storage mould. These high levels are in accordance with the results obtained in others studies that evaluated the microbial quality of vegetable drugs from other authors [7, 8]. Abou-Arab et al. [2] analyzed thirteen samples of medicinal plants and observed that the isolates belonging to different genera were isolated and identified from packed and non-packed samples. The results presented showed that the *Aspergillus* and *Penicillium* genera were more frequently detected and in greater abundance in the packed and non-packed samples than other genera of fungi.

The samples were kept in polyethylene bags, after irradiation and analyzed in the same day (0 day) and after 30 days. The results showed that the dose of 10.0 kGy was the dose required for complete elimination of fungal contamination of medicinal plants on 0 day. The microbial analysis after 30 days showed that no growth of fungi was observed in packed samples of all kind of medicinal plants, comparing with control samples (Table 1).

The levels of water activity (A_w) of samples in table 2 (before and after 30 days of irradiation) showed a decrease of A_w in irradiated samples. The water activity of the control and radiation-processed leaves was in the range of 0.49 to 0.58. There was no evidence of visual colonies on leaves in despite of high counting of moulds in culture medium agar, and this condition could be associated by low A_w of raw material. The minimum A_w for fungal growth is around 0.74 and for xerophytic microbes is 0.61 [9, 10].

The radiation treatment being a nonthermal processing method, does not affect the water content of tea leaves. The storage of inadequately packed or loose-sealed leads to absorption of moisture and to induce fungal growth in samples [11]. Medicinal plants are generally stored and packed in a way to prevent absorption of moisture. Hermetically sealed packets of medicinal plants leaves stored for 30 days (after radiation process) kept good condition.

Table 1. Number of colonies forming units per gram (CFU/g) of total moulds in samples

SAMPLES	DOSE		
	Control (0.0 kGy)	10.0 kGy - 0 day	10.0 kGy - 30 days
Boldo*	1.6 x 10 ⁵	0	0
Espinheira santa*	3.0 x 10 ⁴	0	0
Sene*	2.7 x 10 ⁴	0	0
Chá verde*	3.0 x 10 ³	0	0

*Average of ten samples in three replicates

Table 2. Average of water activity (A_w) of samples.

SAMPLES	A_w^b	A_w^c
Sene ^a	0.58	0.53
Boldo ^a	0.54	0.51
Espinheira Santa ^a	0.52	0.49
Chá verde ^a	0.54	0.50

^aAverage of three replicates;

^b Non-packed samples before irradiation;

^c Packed samples after 30 days of irradiation.

4. CONCLUSIONS

The dose of 10.0 kGy was found to be effective for fungal decontamination of packed leaves of medicinal plants and keep a sterilized condition of products, after 30 days of the radiation treatment. This processing technology improves the microbiological quality and safety of medicinal herbs, as a control of presence of toxigenic moulds (*Aspergillus*, *Penicillium* and *Fusarium*). This treatment can be employed as an effective method for preventing fungal deterioration of medicinal plants during storage. The informations about the levels of fungi in herbs, showed in the present study, provides useful to fiscal authorities, manufactures, consumers, researchers and traders about the importance to improve processing methods to quality control.

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