doi.org/10.15392/2319-0612.2024.2692
2024, 12(4A) | 01-12 | e2692
Submitted: 2024-08-30
Accepted: 2025-03-14

Radiation influence on antioxidant capacity, bioactive compounds and extractability of coffee processing residues

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Abstract: During the agro-industrial process, the complete reuse of waste is an important part of the process's sustainability. However, in most cases, these materials are inadequately discarded, bringing environmental, economic, and social implications. In this context, residue reuse represents an opportunity to develop by-products, in addition to adding value to the raw material. However, conventional treatment processes end up negatively altering the quantity and expressiveness of compounds and nutrients present in this residue. Therefore, ionizing radiation has stood out as a promising technique among the current resources available for residue reuse and microbial load reduction. Thus, the objective of this work is to apply ionizing radiation to coffee processing residues, such as husk and straw, with the aim of preserving and improving the compounds and antioxidant activity. For this purpose, doses of 5 kGy were used on husk and straw residue. The samples were subjected to extraction through physical and chemical processes to obtain an extract from the residue. The extracts were analyzed to identify total phenolic content (TPC), antioxidant activity (ABTS, FRAP, and ORAC), extraction efficiency (EE), and cytotoxicity assays. An improvement in TPC of 26.0% for husk and 19.5% for straw was observed. For antioxidant capacity, an improvement of 14.1%, 16.1%, and 33.3% for husk was observed in ABTS, FRAP, and ORAC analyses, respectively. The 5 kGy dose provided an increase in compound extractability of 26.7% (straw) and 191.9% (husk). In cytotoxicity tests, no significant differences were observed between the irradiated residue and the control. However, an effect related to extract concentration was observed. The irradiation process proved to be a promising technique for agro-industrial residue management. This technique, in addition to promoting already known microbiological benefits, also improved the quantity of compounds, antioxidant activity, and compound extractability. Thus, the present proposal highlights the application of nuclear energy as a viable solution and technological innovation for the reuse of agro-industrial residue.

Keywords: residues, agro-industrial, irradiation, phenolic compounds.

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Influência da radiação na capacidade antioxidante, compostos bioativos e extratibilidade de resíduos do processamento do café

Resumo: Durante o processo agroindustrial, o reaproveitamento integral de resíduos é uma parte importante para a sustentabilidade do processo. Contudo, na maioria dos casos, ocorre o descarte inadequado desses materiais, trazendo implicações ambientais, econômicas e sociais. Neste contexto, o reaproveitamento de resíduos, representa uma oportunidade de desenvolvimento de subprodutos, além de agregar valor à matéria-prima. Entretanto, os processos convencionais de tratamento, acabam alterando de forma negativa a quantidade e expressividade de compostos e nutrientes presentes nesses resíduos. Sendo assim, a radiação ionizante tem se destacado como técnica promissora dentre os recursos atuais disponíveis para reaproveitamento de resíduos e diminuição da carga microbiana. Desta forma, o objetivo desse trabalho consiste em aplicar a radiação ionizante em resíduos do processamento do café, como casca e palha, com a finalidade de preservação e melhoria dos compostos e da atividade antioxidante. Para isso, doses de 5 kGy foram utilizadas no resíduo de casca e palha. As amostras foram submetidas a extração com processos físicos e químicos, para obter um extrato dos resíduos. Os extratos foram analisados para identificação de teor de compostos fenólicos totais (TPC), atividade antioxidante (ABTS, FRAP e ORAC), eficiência de extração (EE) e ensaios de citotoxicidade. Foi observada uma melhora no TPC de 26,0% para a casca e 19,5% para a palha. Para capacidade antioxidante, foi observada uma melhora de 14,1, 16,1 e 33,3% para a casca nas análises ABTS, FRAP e ORAC, respectivamente. A dose de 5 kGy proporcionou um aumento na extratibilidade do composto em 26,7% (palha) e 191,9% (casca). Nos testes de citotoxicidade, não foram observadas diferenças significativas entre o resíduo irradiado e o controle, entretanto um efeito em função da concentração do extrato foi observado. O processo de irradiação se mostrou uma técnica promissora no gerenciamento de resíduos agroindustriais. Essa técnica além de promover benefícios microbiológicos já conhecidos, ainda melhorou a quantidade de compostos, a atividade antioxidante e a extratibilidade dos compostos. Dessa forma, a presente proposta ressalta a aplicação da energia nuclear como uma solução viável e de inovação tecnológica para o reaproveitamento de resíduos agroindustriais.

Palavras-chave: resíduos, agroindustrial, irradiação, compostos fenólicos.

1. INTRODUCTION

The United Nations Organization Agenda 2030 has selected 17 Sustainable Development Goals (SDGs) related to the realization of human rights and the promotion of sustainable development. During the agro-industrial process, the comprehensive reuse of waste is an important part of process sustainability. However, in most cases, there is inadequate disposal of these materials, bringing environmental, economic, and social implications. In this context, waste reuse addresses food waste, representing an opportunity to develop by-products and add value to raw materials. It is also worth noting that agro-industrial waste from the food industry contains significant amounts of nutrients and bioactive compounds (non-nutritional elements such as phenolic compounds, antioxidants, carotenoids, probiotics, fibers, vitamins, and minerals, among others) that can serve as ingredients for functional foods and food additives, and may also find application in the pharmaceutical, chemical, and cosmetic industries. However, conventional treatment processes, such as drying and washing, negatively alter the quantity and expressiveness of compounds and nutrients present in these wastes [1, 2].

In this context, ionizing radiation has emerged as a promising technique among the current resources available for waste reuse, as it is a clean technology that does not generate sub-wastes and does not raise the temperature during processing, the main limitation of conventional methods. With irradiation processing, a new waste management proposal arises with waste reduction, less environmental impact, increased shelf life of foods, and increased bioavailability of bioactive compounds [1].

Thus, one of the agro-industrial wastes generated in large quantities and with low added value is the by-products of coffee bean processing. After harvesting, the coffee fruit, called cherry, when ripe, is placed to dry, a process carried out either naturally (by sunlight) or by industrial dryers with hot air. During this process, the already-dried coffee is separated into three main parts: husk, straw, and green bean (endosperm) [3] The husk is the fruit pulp, corresponding to the outer layer of the fruit, while the straw covers the bean, the latter being the product of interest for coffee beverage production after the roasting process. Both husk and straw are considered waste, sold at negligible values (compared to the bean itself), with some producers using these residues for burning, used in the dryers mentioned above. These by-products still contain a significant amount of compounds that can be reused. Therefore, this work aims to highlight the application of nuclear energy as a viable solution and technological innovation for the reuse of agro-industrial residues from coffee processing, as well as the dissemination of information about the benefits of this technology.

2. MATERIALS AND METHODS

The samples were collected in the region of the city of Espírito Santo do Pinhal in the state of São Paulo, near the border with Minas Gerais. The samples were obtained from a farm that carried out the planting, harvesting, drying, and separation of coffee beans (*Coffea arabica* L., Catuaí Amarelo variety). Husk and straw were collected after the separation process of the green beans. These samples were irradiated at the Radiation Technology Center (CETER - IPEN/SP) using a Cobalt-60 multipurpose irradiator. Harwell Amber 3042 dosimeters and CTA dosimeter were used to control the applied doses and process control.

A dose of 5 kGy (Dose rate 9.111 kGy/h) was used, and control samples (0 kGy - Non-irradiated) were also included. The 5 kGy dose was used based on studies in the literature with agro-industrial residues that achieved good results in the improvement and preservation of compounds, without causing damage or toxicological effects [1,4].

Initially, the samples were crushed, and the generated powder was used in the extraction process employing a mixture of ethanol and water 80:20 (v/v) (at a ratio of 1 g of sample to 10 mL of 80% ethanol) in Falcon tubes at room temperature (25 ºC). Then, this solution was sonicated for 15 minutes, subjected to magnetic stirring (150 rpm) for 15 minutes, centrifuged at 4,000 rpm for 15 minutes at a temperature of 10 ºC, and subsequently filtered through Whatman filter paper Nº. 4 with a vacuum pump. This sequence of processes was carried out in the absence of light whenever possible. After these processes, it was necessary to remove the ethanol by rotary evaporation (136-250 mbar and 40 °C) and lyophilize until the samples were transformed into powder (dry extract).

The extraction yield was calculated by dividing the final dry mass, which is the powdered extract, by the initial dry mass, and the result was expressed as the percentage extraction efficiency (EE). To determine the antioxidant capacity, the ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical method, FRAP (Ferric Reducing Antioxidant Power), and ORAC (Oxygen Radical Absorbance Capacity) were employed, while the bioactive compounds were evaluated by the total phenolic compound content (TPC), with these analyses based on studies from the literature [5,6]. The ABTS radical and ORAC methods were expressed in µmol Trolox equivalents (TE) per 100 g of dry residue extract (µmol TE/100g), on a dry weight basis. For FRAP analysis, it was expressed in µmol of ferrous sulfate (FS) per 100 grams of residue extract (µmol FS/100 grams). The total phenolic compound content (TPC) was expressed in mg of gallic acid equivalents (GAE) per 100 grams of residue extract (mg GAE/100g). The aforementioned procedures were performed in triplicate to ensure data reproducibility and subsequently subjected to analysis of variance using the Tukey test, with a significance level of less than 5%.

During the cytotoxicity assay, the cells (Balb/3T3 cell line (ATCC® CCL-163™)) were trypsinized and seeded in conventional 96-well cell culture plates at a density of $5x10^3$ cells in 100 µL per well. The plates were incubated for 24 hours at 37°C and 5% CO_2. After incubation, dilutions of the sample extracts were added at concentrations of 25, 250, and 2500 µg of dry extract per mL of culture medium.

For reference substances, a 5% PBS solution was used as a negative control (-), and DMSO (dimethyl sulfoxide) diluted to 20% in culture medium was used as a positive control (+). The plates were then incubated for approximately 4 or 24 hours at 37°C and 5% CO_2. After the incubation period, the culture medium was removed from the plates, the wells were washed with PBS without EDTA, and 20 μL of a solution containing MTS (CellTiter 96® AQueous NonRadioactive Cell Proliferation Assay, Promega) at 2 mg/mL in DPBS and PMS (phenazine methosulfate, Sigma-Aldrich) at 0.92 mg/mL in DPBS was added in a 20:1 ratio in RPMI-1640, according to the manufacturer's protocol. After the incubation period (2 hours), absorbance was read at 490 nm by spectrophotometry using the Multiskan equipment (Thermo). For data analysis to determine cell viability, relative viability (percentage of control) was considered, calculated by dividing the mean absorbance of each sample by the mean absorbance of the cell control and multiplying by 100. The graph displaying cell viability information was generated using the GraphPad Prism® statistical program, and statistical analysis was performed using 2-way ANOVA followed by Bonferroni's multiple comparison test. The assays were performed in quadruplicate on the same plate and in duplicate on different days to ensure data reproducibility.

3. RESULTS AND DISCUSSIONS

Table I presents the total phenolic compound content (TPC), antioxidant capacity, and extraction yield for irradiated coffee husk and straw samples with 5 kGy. Initially, a high concentration of compounds present in the husk and straw residues is observed, this result being expected and compatible with other studies in the literature with coffee residues. Despite this result, coffee husk showed a higher concentration than straw in the TPC analysis (33.2%) and ORAC (200%). For the ABTS analysis, there was a slight reduction of 14.1%, while for FRAP, there was no significant difference. The concentration of bioactive compounds in the husk is in agreement with the literature, which has shown that coffee husk presents TPC ranging from 1610 to 15100 mg CAE (chlorogenic acid equivalent)/100g [7]. However, these values can vary according to metabolic, genetic, agronomic, and climatic factors, as well as stress conditions, mainly, climate and soil, fertilization, pathogens and diseases, light intensity, seasonality, among others [8].

An influence was expected depending on the irradiation process on the residues due to the amount of bioactive compounds and antioxidant capacity. Thus, an improvement of 26.0, 14.1, 16.1, and 33.3% was observed for husk in the TPC, ABTS, FRAP, and ORAC analyses, respectively, while for straw, there was only a significant difference for TPC and ABTS, of 19.5 and -16.9%, respectively. The increase in these bioactive compounds, indicated by the TPC concentration, can be justified due to the depolymerization process, which refers to the radiolytic degradation of larger compounds into smaller ones [9]. This phenomenon occurs because the high-energy particles emitted interact directly with the atoms or molecules of the compounds present in the irradiated material, which is an effect of direct ionization. These particles have enough energy to remove electrons from atoms, resulting in the formation of ions. This removal of electrons can break chemical bonds between atoms, causing molecular fragmentation and the formation of new compounds. Another process that can also occur is indirect ionization, in which the particles interact with the surrounding medium, such as air or water, and generate reactive oxygen species and free radicals. These reactive species and free radicals can diffuse into the irradiated material and interact with the compounds present, causing chemical bond breakage and molecular modification. Generally, both processes can occur simultaneously during the irradiation of materials with particle accelerators. Thus, ionizing radiation can physically and chemically influence bioactive compounds and the antioxidant capacity of foods in various ways. Among them, we can mention chemical bond breakage, free radical formation, and molecular structure modifications [10,11].

Table I also observed that the total phenolic compound content was more influenced by irradiation in the husk than in the straw, which can be justified by the higher amount of these compounds, making the influence of irradiation more likely due to the initial composition. The husk also had a more significant result in the extraction capacity of the extract, as observed in Table I. The extraction yield for straw and husk without irradiation did not show a significant difference, but it is observed that the dose of 5 kGy provided an increase in compound extractability by 26.7 and 191.9% for straw and husk, respectively. This effect has already been observed in other studies with residues [9], and this effect can also be justified by the physical effects of irradiation on the structure of compounds, as mentioned earlier.

In general, the influence of irradiation on bioactive compounds and the antioxidant capacity of foods may depend on various factors, including the applied radiation dose, the type of food, the nature of the bioactive compounds present, and post-irradiation storage conditions. Specific studies are needed to better understand these effects and optimize the use of irradiation to preserve and improve the nutritional quality of foods.

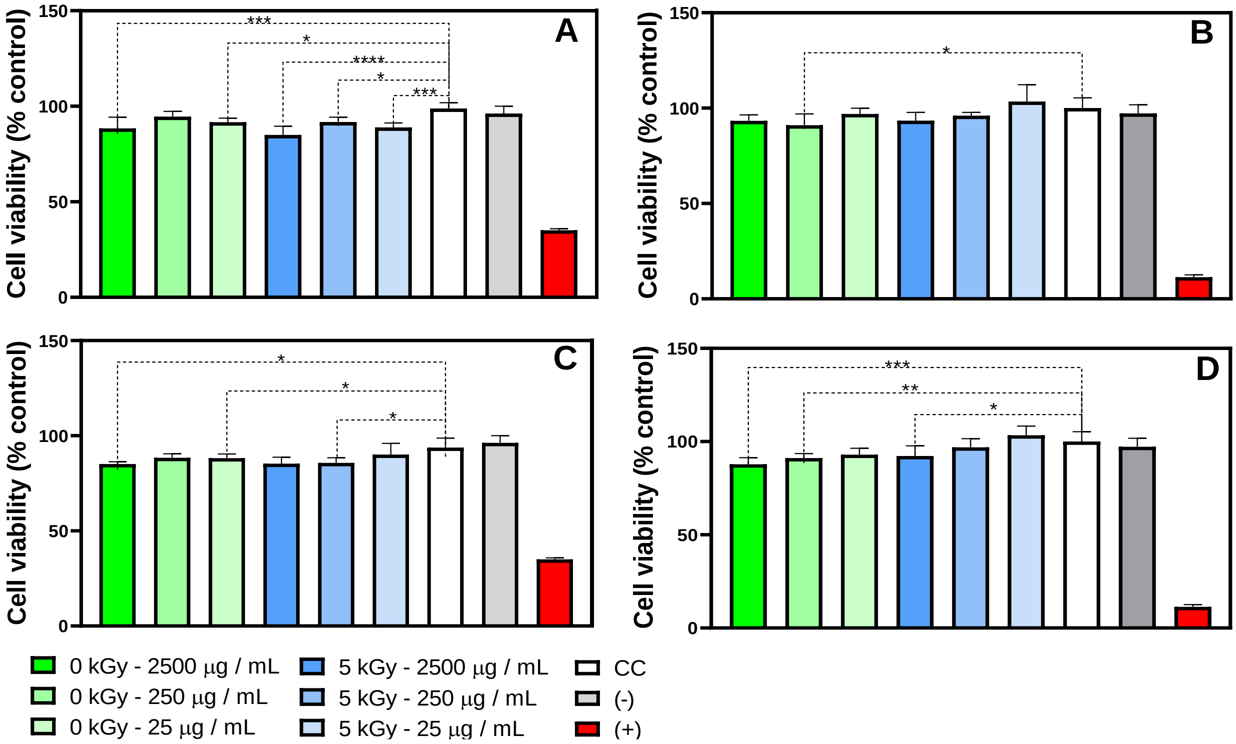
Table 1: Total Phenolic Compounds (TPC), Antioxidant Activity, and Extraction Efficiency (EE) for irradiated husk and coffee straw residues.

Samples / Dose	TPC (mg GAE/100g)	ABTS (µmol ET/100g)	FRAP (10^2 µmol SF/100g)	ORAC (10^{-3} µmol ET/100g)	EE (%)
Straw - 0 kGy	$1239 \pm 11^{d*}$	7.11 ± 0.11^{c}	142 ± 6^{b}	148 ± 9^{c}	11.9 ± 0.2^{c}
Straw - 5 kGy	1481 ± 11^{c}	5.9 ± 0.3^{b}	148.55 ± 0.26^{b}	187 ± 9^{c}	15.0 ± 0.7^{b}
Husk - 0 kGy	1652 ± 26^{b}	6.40 ± 0.08^{a}	156.3 ± 0.5^{b}	324 ± 14^{b}	12.4 ± 0.5^{cb}
Husk - 5 kGy	2081 ± 13^{a}	7.34 ± 0.16^{c}	181 ± 13^{a}	410 ± 30^{a}	36.2 ± 2.2^{a}

*ANOVA and Tukey's test ($p < 0.05$). a, b, c, d, e Different letters in the same column mean that they are statistically different at $p<0.05$.

In vitro cytotoxicity tests define whether a bioactive compound extracted from a waste product can be safely and efficiently assimilated by the body and then used for consumption [12]. In this way, cytotoxicity tests were carried out and the results are presented in Figure 1, for coffee waste extracts (husk and straw) over a period of 4 and 24 hours at different extract concentrations are presented. It is observed that, compared to the cell control (CC), there was a significant difference with the extracts, resulting in a reduction in cell viability. Despite this, it is noted that for the 24-hour assay, this difference in viability decreased for the husk and increased for the straw. When comparing irradiated and non-irradiated extracts at the same concentration, no significant difference was found, indicating that the effect on cell viability is related to the concentration of the extract and the exposure time, not to the effect of the irradiation process. This result suggests that the irradiation process in coffee extracts did not induce any cytotoxic effects. Overall, for the 24-hour tests, no cytotoxic effect was observed at a concentration of 25 μg/mL for either coffee husk or straw.

Figure 1: Cytotoxicity of coffee samples: Husk (A - 4h; B – 24h) and straw (C – 4h; D – 24h). Dose of 0 and 5 kGy, extract concentration of 25, 250 and 2500 μg/mL.



Source: Obtained from the statistical program GraphPad Prism®

4. CONCLUSIONS

The use of agro-industrial residues, such as coffee husk and straw, as raw material for obtaining extracts rich in bioactive compounds with antioxidant action, is a promising strategy that can be further enhanced with the use of ionizing radiation. Additionally, the use of coffee husk and straw helps to reduce the amount of agro-industrial residues and adds value to this specific waste, increasing the possibilities of application in other areas. The total phenolic compound content showed greater influence for the husk than for straw, with irradiation increasing by 26.0 and 19.5%, respectively.

In addition to increasing the quantity of compounds present in the residues, irradiation also has other advantages that further highlight this technology, such as phytosanitary treatment of food, pest and parasite control, reduction of deteriorating organisms, which increases shelf life. Furthermore, food irradiation is a physical process that does not generate heat and helps preserve bioactive compounds present, does not require the use of chemical products or produce chemical residues, and also has significant penetration capacity, promoting a more homogeneous process. Therefore, ionizing radiation technology is a very interesting option for the management and reuse of agro-industrial residues, but it is still a process that requires further investigation to analyze the effects of irradiation on these residues and identify the best irradiation doses that allow the optimal use of these waste extracts.

ACKNOWLEDGMENT

We would like to thank the Comissão Nacional de Energia Nuclear (CNEN), Centro de Tecnologia das Radiações (CTR) and Radiobiology Laboratory of the Centro de Biotecnologia (CEBIO) of the Instituto de Pesquisas Energéticas e Nucleares (IPEN), and the Departamento de Agroindústria, Alimentos e Nutrição (LAN) da Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) for their support with facilities and technical staff.

FUNDING

We appreciate the financial support from the Fundação de Apoio da UFMG (FUNDEP - 28868 - CNEN/IPEN/DPD/EDITAL DE SELECAO DE PROJETOS 06).

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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