



Effect of irradiation on residues from avocado oil processing

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Abstract: In the agro-industrial process, sustainability is an essential characteristic, and the reuse of waste plays an important role in this regard. However, in most cases, these materials are improperly discarded, leading to environmental, economic, and social implications. In this context, there arises a need to utilize waste by extracting nutritional substances that prevent food waste, developing by-products, and adding value to raw materials. It is worth noting that agro-industrial waste from the food industry contains significant amounts of nutrients and bioactive compounds, such as phenolics, antioxidants, carotenoids, fibers, vitamins, and minerals. For example, agro-industrial waste from avocado processing, such as the peel, seeds, and pulp after oil extraction, contains a large amount of bioactive compounds that may have some application. In view of this, the use of ionizing radiation can be considered an innovative technology that has been applied in various processes in the food industry, and it can be used to increase and/or preserve these compounds present in the waste. Thus, the objective of this study was to evaluate the effects of ionizing radiation on waste from avocado oil processing, such as the seed, peel, and extracted pulp. To achieve this objective, the samples were extracted through physical and chemical processes, obtaining a dry extract that was used for analyses of phenolic compound content and antioxidant activity. The results showed that doses of 5 kGy were sufficient to increase the amount of these compounds and antioxidant activity. The peel and seed, which contain the highest amount of compounds, were also the wastes that showed the greatest improvement with the irradiation process. At a dose of 10 kGy, the avocado peel showed significant improvement in both phenolic compound content and antioxidant activity. These results suggest that the use of ionizing radiation technology can be used in the reuse of agro-industrial waste. The results of this study indicated that ionizing radiation did not cause degradation of the compounds, providing strong evidence of one of the benefits of this technique, which is to preserve and maintain food quality.

Keywords: residues, ionizing radiation, avocado, phenolic compounds.



Efeito da irradiação em resíduos do processamento do óleo de abacate

Resumo: No processo agroindustrial, a sustentabilidade é uma característica essencial e o reaproveitamento de resíduos desempenha um papel importante nesse sentido. No entanto, na maioria dos casos, esses materiais são descartados de maneira inadequada, acarretando implicações ambientais, econômicas e sociais. Nesse contexto, surge a necessidade de se aproveitar nos resíduos, substâncias nutricionais que evitam o desperdício de alimentos, desenvolvendo subprodutos e agregando valor à matéria-prima. Vale ressaltar que os resíduos agroindustriais da indústria alimentícia contêm quantidades significativas de nutrientes e compostos bioativos, por exemplo, fenólicos, antioxidantes, carotenoides, fibras, vitaminas e minerais. Como exemplo, podemos mencionar resíduos agroindustriais do processamento do abacate, como casca, sementes e polpa após extração do azeite, contêm grande quantidade de compostos bioativos que podem ser apresentar alguma aplicação. Em vista disso, o uso da radiação ionizante pode ser considerada uma tecnologia inovadora que tem sido aplicada em diversos processo da indústria de alimentos, a qual pode ser empregada com o intuito de aumentar e/ ou preservar esses compostos presentes nos resíduos. Desta forma, o objetivo desse trabalho foi avaliar os efeitos da radiação ionizante em resíduos do processamento do óleo de abacate, como semente, casca e polpa extraída. Para atingir tal objetivo, as amostras foram extraídas por processos físicos e químicos, obtendo um extrato seco que foi utilizado para análises de teor de compostos fenólicos e atividade antioxidante. Os resultados mostraram que doses de 5 kGy foram suficientes para aumentar a quantidade desses compostos e a atividade antioxidante. Casca e semente, onde contêm maior quantidade de compostos, também foram os resíduos que apresentaram maior melhora com o processo de irradiação. Para uma dose de 10 kGy, a casca do abacate apresentou melhora significativa, tanto no teor de compostos fenólicos quanto na atividade antioxidante. Esses resultados sugerem que o uso da tecnologia de radiação ionizante pode ser utilizado no reaproveitamento de resíduos agroindustriais. Os resultados desse trabalho, indicaram que a radiação ionizante não causou degradação nos compostos, fornecendo fortes evidências de um dos benefícios dessa técnica, que é preservar e manter a qualidade dos alimentos.

Palavras-chave: resíduos, radiação ionizante, abacate, compostos fenólicos.

1. INTRODUCTION

During agro-industrial activities, the full reuse of products is an important part of the sustainability of the process. Despite this, in most cases, these materials are discarded or used as animal feed and organic fertilizers, which have low added value when reused. Furthermore, failure to use residue can have environmental and economic implications [1,2].

The use of residue from food processing also contributes to reducing residue and adding value, as these residues can become inputs with high nutritional and functional value, and can be used by the food, pharmaceutical, chemical and cosmetic industries [3]. At the same time, these residues from the food industry present a rich source of nutrients, with significant amounts of bioactive compounds (extra nutritional elements, such as phenolic compounds, sulfur compounds, antioxidants, carotenoids, prebiotics, probiotics, fibers, among others) [4].

Due to the amount of nutrients and the impact of not reusing agro-industrial residue, it is necessary to look for promising techniques that facilitate the success of reusing these residues in other processes. Given this need, the use of ionizing radiation as an auxiliary process for the reuse of agro-industrial residue can be considered a versatile technique, with several technological contributions, but which still needs to be refined and, consequently, be able to expand its applications. Furthermore, the use of ionizing radiation for sustainable development, with a focus on the reuse of residue, is aligned with the Sustainable Development Goals (SDGs) of the United Nations (UN) Agenda 2023. Therefore, the objective of this work was to use ionizing radiation processing to analyze the potential for reusing residue from the avocado industry. To do this, it was necessary to identify the content of phenolic compounds and the antioxidant capacity of the husk, seed and pulp extracted.

2. MATERIALS AND METHODS

The samples were obtained from residue from avocado processing industries with the aim of extracting avocado oil, made with the Avocado variety (grown at Fazenda Vão D'água in Itatinga/SP). The residue used was peel, seed and extracted pulp (which went through the avocado oil extraction process).

The 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 and 10 kGy (Dose rate 9.111 kGy/h) was used, in addition, control samples with a dose of 0 kGy (Non-irradiated) were included. The 5 and 10 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 [5,6].

The samples were extracted with a mixture of ethanol and water 80:20 (v/v) (in a ratio of 1 g of sample to 10 mL of 80% ethanol), in a Falcon tube and at room temperature (25 °C). 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 °C and subsequently filtered through Whatman n° 4 filter paper with pump vacuum. This sequence of processes was carried out in the absence of light, when possible. After these processes, it was necessary to remove the ethanol by roto-evaporation (136-250 mbar and 40 °C) and freeze-dry until the samples were transformed into powder (dry extract). These processes were carried out in triplicate to ensure data reproducibility and subsequently subjected to analysis of variance using the Tukey test, with a significance level lower than 5% ($p > 0.05$).

The procedure for analyzing ABTS, FRAP and total phenolic compound content was based on the methodological procedure described in the study by Silva et al (2023) [7]. To determine the antioxidant capacity, the ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical method was used in a microplate. A solution of ABTS (7 mM) was

mixed with a solution of potassium persulfate (140 mM), reserved for a period of 16 hours in the absence of light at 25 °C. After this period, this final solution was diluted in potassium phosphate (75 mM) until obtaining an absorbance of 0.70 ± 0.02 at 734 nm in a spectrophotometer. To construct the standard curve, Trolox was used as a reference in concentrations ranging from 12.5 to 200 μmol . For reading at 734 nm in the 96-well microplate, 20 μL of the sample or Trolox and 220 μL of the final ABTS solution were added, with a reaction time in the plate of 6 minutes, in the absence of light and at 25°C. The antioxidant capacity results were expressed in μmol Trolox equivalent (TE) per 100 g of dry residue extract ($\mu\text{mol TE}/100\text{g}$), on a dry weight basis.

For FRAP (Ferric Reducing Antioxidant Power) analysis, a FRAP solution was prepared using potassium acetate buffer (0.3 M and pH 3.6), iron chloride solution (20 mM) and TPTZ solution (2,4,6-Tris(2-pyridyl)-s-triazine), in a ratio of 10:1:1 (v/v), respectively. In the 96-well microplate, 20 μL of the sample or standard (ferrous sulfate), 30 μL of distilled water and 200 μL of the FRAP solution were pipetted, which reacted for 8 minutes at 37 °C and read the absorbance at 595 nm. The standard curve was performed at concentrations between 100 and 700 μmol . The reducing power was expressed as μmol of ferrous sulfate (FS) per 100 grams of residue extract ($\mu\text{mol FS}/100 \text{ grams}$).

In the identification of oxygen radical absorbance capacity (ORAC), aliquots of the samples or Trolox at different concentrations were pipetted into a 96-well microplate, followed by a fluorescein solution (508.25 nM) in potassium phosphate buffer (75 mM, pH 7.4) and AAPH solution (76 mM). Fluorescence was monitored every minute for 2 hours, at 485 nm for excitation and 528 nm for emission. The results obtained were expressed in μmol of Trolox equivalents per 100 grams of dry residue extract ($\mu\text{mol TE}/100\text{g}$) [8].

The content of total phenolic compounds was determined by the Folin-Ciocalteu spectrophotometric method, using gallic acid as standard. In a microplate, 500 μL of the sample or gallic acid standard (500 $\mu\text{g}/\text{mL}$) was pipetted, together with 2.5 μL of a 10% (v/v)

Folin-Ciocalteu aqueous solution and after 5 minutes, 2.0 mL of 7.5% (v/v) sodium carbonate solution was added and allowed to react for 40 minutes at 25°C in the absence of light, with absorbance readings at 740 nm. The results were expressed as mg of gallic acid equivalents (GAE) per 100 grams of residue extract (mg GAE/100g).

Reactive oxygen species (ROS), superoxide anion ($O_2^{\bullet-}$), were determined based on the methodology of Silva et al (2023) and Melo et al. (2015) [7,8]. For this, 100 μ L aliquots of the sample or standard at different concentrations, 50 μ L of NBT, 50 μ L of PMS, and 100 μ L of NADH were sequentially pipetted into a 96-well microplate. Absorbance was monitored at 560 nm every 1 minute for 5 minutes of reaction, and the results were expressed as EC_{50} , that is, the concentration ($mg \cdot mL^{-1}$) of the dry extract capable of extinguishing 50% of the superoxide radicals.

3. RESULTS AND DISCUSSIONS

The data on the content of total phenolic compounds (TPC), the free radical scavenging capacity (ABTS), ORAC and the reducing power (FRAP) of the residue samples are presented in Table 1. It is initially observed that the value of total phenolic compounds between samples without irradiation (control - 0 kGy) is approximately 12x greater in the peel than in the seed, while for extracted pulp it is on average 2x more than the seed. This result is consistent with the study by Daiuto et al. (2014), who observed a higher concentration of phenolic compounds in the peel, later in the seed and finally in the pulp [9]. Despite this, the values of phenolic compounds may suffer interference due to metabolic and genetic factors, agronomic and climatic conditions, as well as stress conditions, such as: seasonality, temperature, water availability, ultraviolet radiation, the addition of nutrients, atmospheric pollution, damage mechanics and pathogen attack [9].

Table 1: Antioxidant capacity of seed, extracted pulp and peel samples, at different irradiation doses, determining the content of total phenolic compounds (TPC) of avocado residues, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), elimination of the ABTS radical cation (ABTS•+) and ROS assays and superoxide anion ($O_2^{\cdot-}$).

Samples	TPC (mg GAE/100g)	ABTS ($\mu\text{mol TE}/100\text{ g}$)	FRAP ($\mu\text{mol de FS}/100\text{g}$)	ORAC ($\mu\text{mol TE}/100\text{g}$)	($O_2^{\cdot-}$) ($\text{EC}_{50}\text{ mg}\cdot\text{mL}^{-1}$)
Seed (0 kGy)	7.70 \pm 0.30 ^{g**}	82.50 \pm 2.10 ^b	315.40 \pm 1.80 ^e	0.43 \pm 0.00 ^e	0.22 \pm 0.01 ^{ac}
Seed (5 kGy)	118.80 \pm 1.40 ^c	164.40 \pm 2.60 ^c	481.70 \pm 12.60 ^d	0.88 \pm 0.00 ^d	2.93 \pm 0.20 ^d
Extracted pulp (0 kGy)	16.30 \pm 0.20 ^f	11.70 \pm 0.20 ^a	175.20 \pm 3.20 ^f	0.29 \pm 0.01 ^f	0.60 \pm 0.03 ^b
Extracted pulp (5 kGy)	24.60 \pm 0.80 ^e	6.20 \pm 0.20 ^a	165.10 \pm 2.10 ^f	0.31 \pm 0.00 ^f	0.41 \pm 0.03 ^{bc}
Husk (0 kGy)	94.40 \pm 1.30 ^d	96.90 \pm 2.00 ^b	515.60 \pm 4.20 ^c	0.58 \pm 0.01 ^c	0.07 \pm 0.01 ^a
Husk (5 kGy)	137.80 \pm 2.30 ^b	162.90 \pm 10.10 ^c	877.80 \pm 10.70 ^b	1.04 \pm 0.02 ^b	0.08 \pm 0.01 ^a
Husk (10 kGy)	332.50 \pm 6.30 ^a	265.20 \pm 16.70 ^d	1313.60 \pm 11.90 ^a	1.81 \pm 0.04 ^a	0.58 \pm 0.03 ^b

GAE—gallic acid equivalents; FS—ferrous sulfate; TE—Trolox equivalent. Data were expressed on a dry basis (mass of lyophilized residue extract). The final values are the averages of the triplicates \pm standard deviation.

*Control (Non-irradiated – 0 kGy).

**ANOVA and Tukey test ($p < 0.05$).

For the irradiated samples, there was an improvement in the amount of total phenolic compounds for the three residues analyzed, but with different levels of impacts, a result of the susceptibility to irradiation and the characteristics of each residue. Thus, for a dose of 5 kGy, there was an improvement close to 1442%, 51% and 46%, for seed, extracted pulp and peel, respectively. The improvement in the amount of compounds in the sample has already been verified in another study, where an average increase of 129% in olive pomace was observed for a dose of 5 kGy of gamma radiation, in addition to an increase in antioxidant capacity [10].

This improvement in the concentration of phenolic compounds in irradiated samples can be justified due to the depolymerization process, which refers to the radiolytic degradation of larger compounds into smaller compounds, in addition, this process can increase their extractability [10]. Despite these results, other studies in the literature still have different results, where flour from irradiated citrus fruits preserved the concentration of phenolic compounds and the same result was obtained with pomegranate peel irradiated with 5, 15 and 25 kGy, while for pomace flour apple with 1 kGy, showed better results for antioxidant activity, total

phenolic compounds and some compounds than doses of 2 kGy and the control (0 kGy) [11,12,13]. These results suggest that the effects of irradiation on agro-industrial residue may depend on many factors and that further studies are still needed detailing the effects and correlations with the compounds, in addition to possible applications in reuse.

For the avocado peel, a dose of 10 kGy was used to identify whether the increase in TPC would be proportional or if there would be a decline in its effect. It was observed that doubling the dose of ionizing radiation (from 5 to 10 kGy) promoted an increase of 141.29%. This result suggests that the depolymerization process mentioned earlier can be intensified with the increase in dose. Despite this result, studies in the literature with agro-industrial residues have shown an improvement in TPC up to 10 kGy, but for higher doses, there was a reduction in the total phenolic compounds content [10]. Furthermore, doses above 10 kGy can cause damage to organoleptic properties, especially color and texture [14]. Similar results to TPC were also observed for antioxidant activity (ABTS, FRAP, and ORAC), reinforcing the discussion presented, as can be seen in Table 1.

The avocado seed residue presented a reducing power (FRAP) of 315.4 and 481.7 μmol of FS/100g for 0 and 5 kGy, respectively, corresponding to an increase of 52.7% due to the effect of irradiation, while for husk this increase was 70.2%. The seed was also effective in eliminating the ABTS radical cation, observing a value of 82.5 and 164.4 $\mu\text{mol TE}/100\text{ g}$ for 0 and 5 kGy, respectively, while for the husk it was 96.9 and 162.9 $\mu\text{mol TE}/100\text{ g}$, corresponding to an increase of 99.3 and 68.1%, respectively. Despite these promising results for peel and seed, the extracted pulp residue did not show a significant difference for the ABTS and FRAP analyses.

Na Tabela 1, são apresentados os resultados do radical superóxidos ROS ($\text{O}_2^{\bullet-}$). Essas análises identificam o potencial antioxidante da amostra, devido a transferência de um elétron para o oxigênio molecular, o qual dá origem ao radical superóxido.

It was observed that the irradiated seed showed a higher concentration than the non-irradiated one and also in comparison with the other samples. For the extracted pulp, no significant difference was observed, as well as for the husk. Despite this last result, for the dose of 10 kGy in the husk, an increase in the concentration of superoxide was observed, which can be justified by the effects of irradiation on the compounds within the residues, as mentioned earlier.

It can be seen from the data presented in Table I that before irradiation, the husk was the residue with the greatest potential for reuse, due to the amount of compounds, but after the irradiation process, the seed also became interesting. In this way, these residues that are found in abundance in the avocado processing industry can have their compounds enhanced and thus be reused in various processes with the aid of ionizing radiation.

Despite the promising results obtained in this study, further studies are still needed to understand the mechanisms of action of ionizing radiation and how it affects the compounds present, so that a dose can be determined that achieves maximum efficiency in the irradiation process of the extracts from the residues. Moreover, these residues can undergo various changes according to metabolic, genetic, agronomic, and climatic factors, as well as stress conditions, especially climate and soil, fertilization, pathogens and diseases, light intensity, seasonality, among others [15].

4. CONCLUSIONS

Agro-industrial residue that is normally discarded, often inappropriately and without any pre-treatment, has valuable components that can be used in various industrial applications and can also generate new low-cost products, which increases the added value of these residues. Agro-industrial residues from avocado processing, such as husk, seeds and pulp after extracting the oil, contain a large amount of bioactive compounds that can be

improved through the irradiation process. Doses of 5 kGy were sufficient to increase the amount of these compounds through the depolymerization process, promoting the breakdown of larger compounds into smaller ones due to the physical effect of irradiation. Peel and seed, where they contain the greatest amount of compounds, were also the residues that showed the greatest improvement with the irradiation process. For a dose of 10 kGy, the avocado peel showed a significant improvement, both in the content of phenolic compounds and in the antioxidant activity.

In this way, this work, as well as others in the literature, contribute to strengthening the importance of applying this technology in the processing of agro-industrial residue with the aim of reusing it, avoiding residue, inappropriate disposal and adding value. Furthermore, it is worth highlighting that food irradiation has other advantages in eliminating pathogens and reducing microbiological load, which only strengthen the benefits of using this process. In general, the irradiation of agro-industrial residue indicates that it is a promising technique, but additional studies are needed to better understand the effects of ionizing radiation on residue and the possible applications for this residue.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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