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Effect of irradiation on residues from avocado oil processing

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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 waste can have environmental and economic implications [1,2].
The use of waste from food processing also contributes to reducing waste and adding value, as these wastes 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 wastes 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 waste, it is necessary to look for promising techniques that facilitate the success of reusing these wastes in other processes. Given this need, the use of ionizing radiation as an auxiliary process for the reuse of agro-industrial waste 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 waste, 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 waste from the avocado industry. To do this, it was necessary to identify the content of phenolic compounds and the antioxidant capacity of the peel, seed and pulp extracted by the ABTS and FRAP method.

2. Methodology

The samples were obtained from waste 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 waste 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) in electron beams - Dynamitron (IBA Industrial Inc., Edgewood, NY, USA). An average dose of 5 kGy was used, in addition, control samples with a dose of 0 kGy were included. Harwell Amber 3042 dosimeters and CTA dosimeter were used to control the applied doses and process control.
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 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 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%.

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) [5]. 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 (µmol TE/100g), 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 (µmol FS/100 grams).

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 µg/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).

3. Results and Discussion

The data on the content of total phenolic compounds, the free radical scavenging capacity (ABTS) and the reducing power (FRAP) of the waste samples are presented in Table I. 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 [6]. 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 [6].

For the irradiated samples, there was an improvement in the amount of total phenolic compounds for the three wastes analyzed, but with different levels of impacts, a result of the susceptibility to irradiation and the characteristics of each waste. 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 [7]. 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 [7]. 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) [8,9, 10]. These results suggest that the effects of irradiation on agro-industrial waste 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.

Table I: Antioxidant capacity of seed, extracted pulp and peel samples, at different irradiation doses, determining the content of total phenolic compounds of avocado residues in mg of gallic acid equivalent (GAE), ferric reducing antioxidant power (FRAP) in μmol of ferrous sulfate (FS) and elimination of the ABTS radical cation (ABTS•+) in μmol equivalent to Trolox (ET).

Samples	**Dose (kGy)**	**Total phenolics (mg GAE/100g)**	**ABTS (μmol ET/100 g)**	**FRAP (μmol de FS/100g)**
Seed	0*	7,7 ± 0,3 f**	82,5 ± 2,1 b	315,4 ± 1,8 d
Seed	5	118,8 ± 1,4 b	164,4 ± 2,6 c	481,7 ± 12,6 c
Extracted pulp	0	16,3 ± 0,2 e	11,7 ± 0,2 d	175,2 ± 3,2 e
Extracted pulp	5	24,6 ± 0,8 d	6,2 ± 0,20 d	165,1 ± 2,1 e
Pell	0	94,4 ± 1,3 c	96,9 ± 2,0 a	515,6 ± 4,2 b
Pell	5	137,8 ± 2,3 a	162,9 ± 10,1 c	877,8 ± 10,7 a

*Control (Non-irradiated).

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

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 pell 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 μmol ET/100 g for 0 and 5 kGy, respectively, while for the pell it was 96.9 and 162.9 μmol ET/100 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.
It can be seen from the data presented in Table I that before irradiation, the pell 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.

4. Conclusions

Agro-industrial waste 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 wastes. Agro-industrial residues from avocado processing, such as pell, 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. 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 waste with the aim of reusing it, avoiding waste, 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 waste indicates that it is a promising technique, but additional studies are needed to better understand the effects of ionizing radiation on waste and the possible applications for this waste.

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