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Ionizing radiation from Cobalt-60 in coffee beans (*Coffea arabica*): physical and chemical evaluation

Radiación ionizante de Cobalt-60 en granos de café (*Coffea arabica*): evaluación física y química

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ABSTRACT:

This study aims/seeks to test the difference between packaging, different storage times and different doses of Co-60 gamma radiation in the chemical composition of coffee beans. The present experiment was carried out at the Laboratory of Bromatology and Water at the Federal Institute of Education, Science and Technology of the South of Minas Gerais - Campus Muzambinho, from August 2018 to July 2019. Coffee beans from the cultivar Catucaí IAC 144 were used, which were provided by the Coffee Industrialization and Quality Laboratory of IFSULDEMINAS - Campus Muzambinho. The treatments were two types of packaging (vacuum and common), three storage times (30, 60 and 90 days), and four Co-60 radiation doses with (0, 2, 4, 8 kGy). Analysis were performed to chemical composition, phenolic compounds, and antioxidant activity. The results obtained were submitted to variance analysis by F test ($p < 0.05$), followed by a Scott Knott test at 5. probability to compare the means. According to the results found, it is concluded that the coffee stored in vacuum packaging presents a better preservation of phenolic compounds. Cobalt-60 Gamma Irradiation increased the antioxidant properties of coffee, in doses of 4 and 8 kGy, proving to be an interesting option in harvesting the beans.

KEYWORDS: Food Preservation, Post-Harvest, Centesimal Evaluation.

RESUMEN:

Este estudio tiene como objetivo / busca probar la diferencia entre envasado, diferentes tiempos de almacenamiento y diferentes dosis de radiación gamma Co-60 en la composición química de los granos de café. El presente experimento se llevó a cabo en el Laboratorio de Bromatología y Agua del Instituto Federal de Educación, Ciencia y Tecnología del Sur de Minas Gerais - Campus Muzambinho, de agosto de 2018 a julio de 2019. Se utilizaron granos de café del cultivar Catucaí IAC 144, que fueron proporcionados por el Laboratorio de Industrialización y Calidad del Café de IFSULDEMINAS - Campus Muzambinho. Los tratamientos fueron dos tipos de envasado (vacío y común), tres tiempos de almacenamiento (30, 60 y 90 días) y cuatro dosis de radiación Co-60 con (0, 2, 4, 8 kGy). Se realizaron análisis de composición química, compuestos fenólicos y actividad antioxidante. Los resultados obtenidos se sometieron a análisis de varianza mediante la prueba F ($p < 0.05$), seguida de una prueba de Scott Knott al 5% de probabilidad para comparar las medias. De acuerdo con los resultados encontrados, se concluye que el café almacenado en envasado al vacío presenta una mejor conservación de los compuestos fenólicos. La Irradiación Gamma Cobalto-60 incrementó las propiedades antioxidantes del café, en dosis de 4 y 8 kGy, demostrando ser una opción interesante en la cosecha del grano.

PALABRAS CLAVE: Conservación de alimentos, Post-cosecha, Evaluación Centesimal.

INTRODUCTION

Currently, the foreign market is increasingly demanding, regarding quality and food safety (Martinelli Júnior, 2015). Brazil has coffee as one of its main commodities. The growing consumption of coffee and the consequent effects on human health developed interest in the scientific community, which led to the development of studies related to the biological activity of the bean and the constituents of coffee, as it is very common in several types of drinks (Lima et al., 2010).

The chemical composition values of raw coffee beans may vary due to the variety of coffee, place of harvest and processing techniques (Saldanha et al. 1997). According to Ferreira (2010), he states that the quality of Arabica coffee is closely linked to the physical-chemical constitution of raw beans.

The inappropriate harvesting and post-harvesting techniques can cause damage to the coffee fruits. Once the membranes of the cell wall are affected, it occurs the deterioration of the beans in an expressive manner, causing loss of quality (Borém et al, 2008). The storage of raw grains, which have already been processed, has as main objective to maintain the quality of the product between the process of harvest and agricultural commercialization, allowing the adequate distribution and markets supply (Borém et al, 2007). Generally, the storage process in Brazil is carried out by cooperatives and coffee warehouses, for greater producers security. In addition to that there is the quality of the storage service, the use of qualified labor to carry out the processes, and the loyal market for the grains export.

In the discovery of irradiation and atomic theory, the field of applications has been gaining prominence, for example, in medicine, industry and agriculture (Xavier et al. 2007). Food irradiation is a preventive measure of food security and it consists of a process in which, packaged or bulk food, is subjected to a controlled amount of radiation in order to inhibit sprouts development, delay fruit ripening, reduce microbial load, reduce populations of *pathogenic microorganisms*, sterilization, disinfection of grains, cereals, fruits and spices. Sterilization by radiation provides a pathogen-free diet. These pathogens can be found on the surface or inside of the food due to inadequate hygiene or natural decomposition process (Rocha et al., 2007).

In Brazil, the Resolution RDC nº 21 of 01/26/2001 approved the "Technical Regulation for Food Irradiation" that allows the irradiation of any food, with the proviso that the maximum absorbed dose is lower than that compromised as the characteristics functional and/or sensory properties of the food. And that the minimum absorbed dose is sufficient to achieve the intended objective (Brasil, 2001).

In this context, the present study/work aims to test different packages, storage times and different radiation dose with Co-60 in the storage of coffee and its influence on the physical and chemical composition.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Bromatology and Water at the Federal Institute of Education, Science and Technology of the South of Minas Gerais - Campus Muzambinho, from August 2018 to July 2019. It was used 4.8 kilograms of coffee beans, *Coffea Arabica* L., cv. Catuaí Vermelho - IAC 144 granted from the Industrialization and Coffee Quality Laboratory of IFSULDEMINAS - Campus Muzambinho in the municipality of Muzambinho - Minas Gerais (Figure 1).



FIGURE 1
Location of the city where this research was carried out

The treatments consisted of the evaluation of two types of packaging, four storage times and four irradiations doses. The samples were prepared and separated in 200 g packages, totaling 2.4 kg coffee samples,

where 12 packages of 200 g were vacuum-packed, and 12 packages of 200 g were packed in common packaging, followed by the irradiation.

The samples were irradiated at IPEN - Institute of Energy and Nuclear Research - University of São Paulo. A multi-purpose irradiator was used under a dose rate of 5 kGy/hour, where the whole grains were subjected to gamma radiation of Cobalt-60. The irradiation treatments were: non-irradiated control, 2kGy, 4kGy and 8kGy.

After the irradiation process, the samples were stored at room temperature. The coffee quality assessments were carried out on the physical and chemical parameters. It started off with the pre-drying of the samples and evaluations in the periods of 0, 30, 60 and 90 days. The other analysis were performed at the Laboratory of Bromatology and Water at IFSULDEMINAS-Campus Muzambinho.

Chemical analysis of moisture, ashing, ether extract and protein were performed according to the methodology described by (AOAC, 1995). To determine the moisture content it was used the gravimetric analysis, in which the samples were dried in an oven heated to a temperature of 105 °C, *until it reaches constant weight*.

The fixed mineral material (ash) was determined by incinerating the samples in a muffle furnace at a high temperature of 550-600 °C for 4 hours.

Total nitrogen content was determined by the Micro-kjeldahl method. The protein content was determined by multiplying the total nitrogen content by the single factor 6.25. The ether extract was determined using the Soxhlet extractor and ethyl ether for lipid extraction.

The crude fiber content was determined by the gravimetric analysis of Kamer and Ginkel (1952), using nitric, sulfuric and trichloroacetic acid. The percentage of carbohydrate were calculated by subtracting the lipids, proteins, moisture and ash from one hundred (Equation 1) (IAL, 2008).

$$\% \text{ Carbohydrates} = (U - P - C - L) \quad (\text{Eq 1})$$

Where: P = protein, L = Lipids, C = Ashes, U = Moisture.

The determination of total phenolic concentration was determined using the methodology performed by Swain and Hillis (1959). It was conducted an extraction with methanol, added Folin-Denis reagent, saturated with sodium carbonate solution, and the absorption is read at 660 nm in a spectrophotometer. The results were expressed in $\mu\text{g of g}^{-1}$ sample.

The antioxidant capacity of the coffee cultivar extract was measured according to the method developed by Brand-Williams, Cuvelier and Berset (1995), using 1,1-diphenyl-2-picrylhydrazil (DPPH). Antioxidant activity was expressed as a percentage inhibition: $\% \text{ antioxidant activity} = (\text{Abs of control} - \text{Abs of sample}) * 100 / \text{Abs of control}$. Absorbance was measured at 517nm (SINGH; MURTHY; JAYAPRAKASHA, 2002).

In the experiment, three replicates per treatment were performed. The results obtained were submitted to variance analysis by F test ($p < 0.05$), followed by a Scott Knott test at 5% probability to compare the means. All analysis were performed using the Sisvar software (FERREIRA, 2000).

RESULTS AND DISCUSSION

The centesimal composition of the two packages is shown in Table 1. It can be seen that the packages showed differences in moisture and crude fiber contents, and the common packaging provided less moisture and more crude fiber than vacuum packaging.

TABLE 1

Centesimal composition in fresh basis (g.100g⁻¹ of sample) of the two packages analyzed in the experiment (common and vacuum packaging), Muzambinho, 2019

Packages	Moisture	Ashes	Proteins	Lipids	Crude fiber	Carbohydrates
Vacuum	3,96 ± 0,3 ^{1 a2}	3,35 ± 0,9 ^a	11,25 ± 0,8 ^a	4,34 ± 0,7 ^a	11,10 ± 0,8 ^b	66 ³
Common	3,02 ± 0,4 ^b	3,15 ± 0,3 ^a	11,06 ± 0,5 ^a	4,45 ± 0,4 ^a	13,62 ± 1,0 ^a	64,7 ³

1 média desvio padrão, 2 médias com letra(s) diferente(s) na vertical diferem significativamente em nível de p≤0,05. 3 médias obtidas por diferença.

Table 2 shows the data relative to the maintenance of phenolic compounds and antioxidant activity (DPPH) in both packages. The use of vacuum packaging provided better antioxidant activity compared to common packaging in coffee samples. This fact corroborates with that described by Reis et al. (2013), who states that the antioxidant action of coffee is strongly influenced by the type of packaging used.

TABLE 2

Total phenolic compounds and antioxidant activity (DPPH) in fresh basis (g.100g⁻¹ of sample) from the two packages analyzed in the experiment (common and vacuum packaging), Muzambinho, 2019

Packages	Phenolic Compounds	DPPH
Vacuum	115,00 ± 171,0 ^{1 b2}	57,73 ± 2,5 ^{1 a2}
Common	189,06 ± 237,7 ^a	64,88 ± 3,2 ^a

1 média desvio padrão. 2 médias com letra(s) diferente(s) na vertical diferem significativamente em nível de p≤0,05.

Nascimento (2006) observed a progressive antioxidant activity decreasing with roasting. In contrast to the result obtained in this study, where the increase in radiation doses promoted an increase in antioxidant activity. Castillo et al. (2002), evaluated the antioxidant activity of coffee submitted to different degrees of roasting and pointed out a decrease in the content of chlorogenic acids and an increase in the antioxidant activity of melanoidins in light and medium roast coffee, in comparison with fresh coffee. Araújo (2007), on the other hand, says that during the roasting process, the bonds between the phenolic compounds and

the molecules linked to them are broken, which gives the resulting compounds a structure with greater antioxidant capacity. Thus, increases in radiation doses would be more beneficial to human health and more effective in protecting cells from oxidative stress.

According to Barros et al. (2015), the maintenance of the content of phenolic compounds is very important due to its medicinal properties, being responsible for the neutralization of free radicals, in addition to anticarcinogenic and antiteratogenic effects. In this sense, vacuum packaging provided better maintenance of the medicinal properties of coffee beans. According to Farah and Donangelo (2006), lower levels of the phenolic fraction of Arabica Coffee seem to be one of the explanations given for its superior quality in the drink. The centesimal composition of coffee beans subjected to different radiation doses for cobalt-60 can be seen in Table 3. For the ash, protein and lipid parameters, there was no significant difference in the different radiation doses evaluated.

TABLE 3
Centesimal composition in fresh basis ($\text{g} \cdot 100\text{g}^{-1}$ of sample) at the radiation doses evaluated in the experiment (control, 2, 4 and 8 kGy), Muzambinho, 2019

DOSES (KGY)	Moisture	Ashes	Proteins	Lipids	Crude fiber	Carbohydrates
0	$3,72 \pm 0,4^1$ a^2	$3,40 \pm 0,7^a$	$11,28 \pm 0,8^a$	$4,79 \pm 0,9^a$	$12,92 \pm 0,4^a$	$63,89^3$
2	$3,40 \pm 0,6^a$	$3,25 \pm 0,2^a$	$11,26 \pm 0,6^a$	$4,54 \pm 0,6^a$	$10,08 \pm 0,9^b$	$67,47^3$
4	$3,43 \pm 0,2^a$	$3,36 \pm 0,4^a$	$11,12 \pm 0,7^a$	$4,25 \pm 0,6^a$	$10,47 \pm 0,3^b$	$67,37^3$
8	$2,60 \pm 0,4^b$	$3,35 \pm 0,3^a$	$10,96 \pm 0,3^a$	$3,98 \pm 0,7^a$	$10,60 \pm 0,6^b$	$68,61^3$

1 média desvio padrão. 2 médias com letra(s) diferente(s) na horizontal diferem significativamente em nível de $p \leq 0,05$. 3 médias obtidas por diferença

The moisture content was reduced with the radiation treatment in the dose of 8 kGy. Several studies have shown that the irradiation process (8kGy) alters the physicochemical properties of legume grains (DOGBEVI et al, 2000), and also changes the properties of starch in doses higher than 2.5 kGy (RAYAS-DUARTE & RUPNOW, 1993; RAYAS-DUARTE et al., 1987). Although these studies were conducted with different species, it is possible that these changes may also have occurred in the coffee samples used in the present study.

All irradiated samples had a reduced crude fiber content compared to the non-irradiated control. Studies show that irradiation of plant-derived food can cause a reduction in oligosaccharides, possibly due to denaturation and aggregation reactions (RAO & VAKIL, 1983).

Table 4 shows the data related to the maintenance of phenolic compounds and antioxidant activity (DPPH) of coffee beans submitted to different absorption doses of gamma irradiation from Cobalt-60. A significant increase in the antioxidant activity (DPPH) of the coffee bean was noted with the doses of 4 and 8 kGy of Cobalt-60. During grain storage at room temperature, it occurs oxidation that darkens the grain and makes it less attractive to consumers. The maintenance of the antioxidant activity allows storage for a longer period of time on the shelves. Similar results were obtained by Carvalho (1991) who concluded that irradiation, in addition to maintaining nutritional quality, promotes increased grain life.

TABLE 4

Total phenolic compounds and antioxidant activity (DPPH) in fresh basis (g.100g⁻¹ de of sample) at the radiation doses analyzed in the experiment (control, 2, 4, and 8 kGy), Muzambinho, 2019

DOSES (KGY)	Phenolic Compounds	DPPH
0	141,36 ± 198,2 ^{1a2}	48,00 ± 2,6 ^{1 c2}
2	153,78 ± 210,7 ^a	52,82 ± 2,0 ^b
4	143,73 ± 219,3 ^a	57,38 ± 2,9 ^b
8	169,26 ± 247,0 ^a	63,86 ± 3,2 ^a

1 média desvio padrão. 2 médias com letra(s) diferente(s) na horizontal diferem significativamente em nível de p≤0,05.

There was a correlation between the increase in total phenolic compounds and the increase in irradiation with Cobalt-60. According to Wettasinghe e Shahidi (1999) phenolic compounds are secondary natural metabolites of plants and are formed under stress conditions such as infections, injuries and oxidation.

The proximate composition of coffee beans submitted to different storage times can be seen in Table 5. Regarding the storage time, there was an increase in humidity, and after 60 days there was no change in the increase. The amount of ash and protein decreased with 90 days of storage, and the amount of crude fiber was decreasing at all evaluations in different timing.

TABLE 5
Centesimal composition in fresh basis ($\text{g} \cdot 100\text{g}^{-1}$ of sample) in the evaluated treatments (30, 60 and 90 days), Muzambinho, 2019

Treatment (days)	Moisture	Ashes	Proteins	Lipids	Crude fiber	Carbohydrates
30	$2,94 \pm 1,3^1$ b2	$3,54 \pm 0,1^a$	$11,53 \pm 0,6^a$	$4,19 \pm 0,2^a$	$15,13 \pm 0,6^a$	$62,67^3$
60	$3,25 \pm 0,3^a$	$3,25 \pm 1,3^a$	$11,12 \pm 0,6^a$	$4,96 \pm 0,7^a$	$12,81 \pm 0,7^b$	$64,61^3$
90	$3,54 \pm 2,0^a$	$2,94 \pm 0,1^b$	$10,82 \pm 0,6^b$	$4,02 \pm 0,4^a$	$9,14 \pm 0,4^c$	$69,54^3$

1 média desvio padrão. 2 médias com letra(s) diferente(s) na horizontal diferem significativamente em nível de $p \leq 0,05$. 3 médias obtidas por diferença.

For the lipid variable, there was a significant increase in the mean storage time of 60 days compared to the other treatments. However, for the crude fiber there was a significant decrease in the means between the time of 30, 60 days and 90 days of storage. During the storage Kon (1968) mentions that several reactions occur inside the seed that can cause an increase or decrease in nutritional parameters of the grain.

Table 6 shows the data related to the maintenance of phenolic compounds and antioxidant activity (DPPH) of coffee beans submitted to different storage times. It is possible to notice that the time promoted a significant reduction in the antioxidant activity of coffee. It is known that the increase in temperature can accelerate chemical reactions, or even in this case, it can cause loss of antioxidant activity in samples subjected to heat. The samples were stored in a storage room at 25°C without relative humidity control. Some authors also state that the stability of antioxidant compounds also decreases with exposure to light and oxygen, which could explain the result found in the present study (CUPPET, SCHNEFF & HALL, 1997).

TABLE 6
Total phenolic compounds and antioxidant activity (DPPH) in fresh basis (g.100g⁻¹ of sample) in diferente storage time (30, 60 and 90 days), Muzambinho, 2019

Time (days)	Phenolic Compounds	DPPH
30	130,05 ± 129,5 ^{1 a2}	62,59 ± 5,0 ^{1 c2}
60	150,77 ± 161,7 ^a	55,13 ± 2,2 ^b
90	175,58 ± 181,2 ^a	50,28 ± 2,5 ^a

1 média desvio padrão. 2 médias com letra(s) diferente(s) na vertical diferem significativamente em nível de p≤0,05.

Rodrigues (2012) in a comparative study of coffee crops observed that low levels of total phenolic compounds can be associated with storage, roasting point and maturation of coffee beans. The author also observed that coffee crops that have a higher amount of green fruits tend to have a higher phenolic content. Rodarte et al. (2009) state that the roasting point is an important factor for concentrations of phenolic compounds in coffee beans, whose darker roasts tend to degrade more these compounds when compared to lighter roasts. Factors such as the type of grain used, production, harvesting and processing until the final product can contribute to variations in the content of bioactive constituents, especially phenolic compounds. These factors can determine samples with better functional potential for human health, considering the inherent biological properties of polyphenols.

CONCLUSIONS

Coffee stored in vacuum packaging showed better maintenance of the phenolic compounds. Cobalt-60 radiation increased the antioxidant properties of coffee beans at doses of 4 and 8 kGy.

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