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MINIMALLY PROCESSED MIXED SALAD SUBMITTED TO GAMMA RADIATION: EFFECTS ON BIOACTIVE COMPOUNDS

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ABSTRACT

High consumption of fruits and vegetables has been associated with a lowered incidence of oxidative stress-related diseases due to the presence of bioactive structures. Minimally processed products are a growing segment in food retail establishments because it is associated with practicality and convenience without significantly altering fresh-like characteristics. Low-dose of gamma radiation in combination with minimal processes has shown to be a promising strategy for extending shelf life and maintaining the organoleptic quality of fruits and vegetables. The objective of this study was to evaluate the levels of phenolic compounds, flavonoids, proanthocyanidins and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical scavenging and Oxygen Radical Absorbance Capacity (ORAC) method in minimally processed mixed salad before and after different radiation doses. Samples of minimally processed mixed salad (with green and red cabbage and carrot) were purchased at local supermarket and irradiated with doses of 0.5, 1.0, 2.0 and 3.0 kGy. Phenolic compounds, flavonoids, proanthocyanidins and antioxidant activity by DPPH• and ORAC were analized on the same extract prepared with MeOH. The results showed that bioactive compounds levels and antioxidant activity decreased significantly ($p<0.05$) with an increasing on radiation dose. Gamma-rays may affect these compounds and can cause degradation or oxidation, which can explain the drop on levels. Although the radiation has affected the bioactive contents, the process seems to be interesting to maintaining organoleptic characteristics and provide microbiological security at doses up to 2.0 kGy, according to studies conducted by our research group.

1. INTRODUCTION

Since the 1970's, minimally processed or ready-to-eat products are marketed in the USA, unlike Brazil, which became commercially available only in the past two decades. Nowadays, they represent an important piece of national food market, accounting for 10%–13% of total sales in large supermarket chains. The main reason about a growing consumption is based to fulfill consumer demands for healthy, palatable and easy to prepare foods [1-2].

This kind of product can be obtained from fresh products through selection, washing, peeling, cutting, sanitization, rinsing, drying and packaging [1-3]. During the process, the cutting operations involved means that cells are disrupted, which causes the release of enzymes and substrates, and can increase oxidative enzyme-catalysed processes, which can modify some structures and alter nutritional properties on storage [4]. To minimize damage, several

technologies, including antioxidant treatment, modified atmosphere packaging, refrigeration and irradiation, can be applied in order to extend shelf life and preserve nutritive and sensorial properties of minimally processed products, beyond to provide microbiological safety [5-6].

Irradiation with ionizing radiation, such as gamma radiation, is well established as a physical and non-thermal method of preservation (cold-pasteurization) of food at or near ambient temperatures [7-9]. Low dose of γ-irradiation in combination with other processes, such as minimally processes, has shown to be a promising technique for extending the shelf life and maintaining organoleptical quality of fruits and vegetables, despite little information is available on nutrient stability or on the effectiveness of postharvest treatments on their nutritive value retention [10-11].

It is well known that fresh fruits and vegetables contain nutritional and healthful constituents, also a range of micronutrients including minerals, vitamins, phytochemicals such as carotenoids and polyphenols, and dietary fibers. Epidemiological studies indicated a relation between the frequent consumption of fruits and vegetables and reduce the incidence of chronic diseases, due to the presence of bioactive compounds, such as phenolic compounds. In recent decades, polyphenols have attracted considerable interest in food technology research owing their antioxidant properties [4, 12-15].

According to this fact, the application of preservation treatments can be an alternative to offer a quality product with extended shelf life. Meanwhile there is a growing scientific interest in the influence of irradiation processes on nutritional quality, mainly antioxidant capacity and the compounds responsible for such activity. Therefore, the objective of this study was to investigate the effect of gamma irradiation on levels of bioactive compounds and antioxidant activity, in minimally processed mixed salad.

2. MATERIALS AND METHODS

2.1. Minimally Processed Samples

Samples of minimally processed mixed salad, composed by green and purple cabbage and carrot, were purchased at local supermarket in the city of São Paulo, Brazil. The material was available in perforated plastic material packages with 250 g, under refrigeration and all the samples were kept at 4ºC before irradiation.

2.2. Irradiation

The mixed salads were irradiated in a Multipurpose Cobalt-60 Irradiator Source (IPEN, São Paulo – Brazil) and were divided in five treatments: control and doses of 0.5, 1.0, 2.0 and 3.0 kGy. Gammachrome YR Bath 530 nm dosimeters were used for the measurement of radiation dose.

2.3. Extraction of Bioactive Compounds

For phenolic compounds, flavonoids, anthocyanins and antioxidant activity analysis, one gram of freeze-dried samples was homogenized two times with 10 mL and once with 5 mL of methanol:water:acetic acid (70:29.5:0.5, v/v/v) and sonicated for 15 min. The suspensions were centrifuged at 7000 rpm for 10 min and 4ºC and the supernatants collected. Extracts were

combined and brought to 25 mL with methanol/acid acetic solution and extracts were stored at -20ºC until analysis [16].

2.4. Determination of Phenolic Contents

Briefly, appropriate dilutions of extracts were oxidized with Folin Ciocalteau reagent, and the reaction was neutralized with 0.5 M NaOH. The absorbance of the resulting blue color was measured at 760 nm after 2 h using microplate reader in UV-Visible spectrophotometer mode (Synergy HT, BioTek Instruments, Inc., USA). Total phenolics were quantified by using a gallic acid standard curve (1.5 to 11.25 µg/mL; r =0.99). Results were expressed in µg of gallic acid equivalents per g of salad (µg of GAE/g sample). Analyses were made in triplicate and results were expressed on dry basis [17].

2.5. Determination of Flavonoids

Total flavonoid content was determined using a colorimetric method [18]. Briefly, a dose of 0.5 mL of the extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, followed by adding 75 µL of a 5% (w/v) $NaNO_2$ solution. After 6 min, 150 µL of a 10% (w/v) $AlCl_3{\cdot}6H_2O$ solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1 M NaOH. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using microplate reader in UV-Visible spectrophotometer mode (Synergy HT, BioTek Instruments, Inc., USA). The results were calculated and expressed as µg of (+)-catechin equivalents (µg of CAE/g sample) using the calibration curve of (+)-catechin. Linearity range of the calibration curve was 7.5 to 25 µg/mL (r = 0.99). The extraction was conducted in triplicate and results were expressed on dry basis [18].

2.6. Determination of Proanthocyanidins

To 0.5 mL of the extract or (+)-catechin standard solution, 1.5 mL of a 4% (w/v) methanol vanillin solution and 0.75 mL of concentrated hydrochloric acid were added. The mixture stood for 15 min, and the absorption was measured at 500 nm against methanol as a blank using microplate reader in UV-Visible spectrophotometer mode (Synergy HT, BioTek Instruments, Inc., USA). The amount of proanthocyanidins was calculated and expressed as µg of catechin equivalents (µg of CAE/g sample) using the calibration curve of (+)-catechin. Linearity range of the calibration curve was 5 to 20 µg/mL (r =0.99). For each specific sample, triplicate extractions were performed and used for analyses [18].

2.7. Determination of Antioxidant Activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH•) Radical

Radical scavenging activity was determined using DPPH• radical [19]. Briefly, stock solution of DPPH• was mixed to the extract samples in a 96-well microplate reader. The absorbance of the remaining DPPH• was determined at 517 nm against a blank (methanol 80%) using a microplate reader (Synergy HT, BioTek Instruments, Inc., USA). To calculate the percentage of radical neutralization, Trolox was used to standard curve (12.5 to 100 µM). Results were expressed in µmols of Trolox equivalents per g of salad in dry base.

2.8. Determination of Antioxidant Activity by Oxygen Radical Absorbance Capacity (ORAC)

The analytical procedure of Huang and others [20] was followed for the ORAC assay. Briefly, 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was used as a peroxyl radical generator, Trolox as a standard, and fluorescein as a fluorescent probe. Filters were used to select an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Twenty-five μL of diluted sample, blank, or Trolox calibration solution (0–100 μM) were added to the designated wells of a 96-wells black plate and mixed with 150 μl of 4 nmol fluorescein. The mixture was incubated at 37 ºC for 30 min in microplate reader (Synergy HT, BioTek Instruments, Inc., USA) before injection of 25 μL AAPH solution (153 mM). The fluorescence was measured every 1 min for 1 h. The final ORAC values were calculated using the net area under the decay curves.

2.9. Moisture

The moisture content was gravimetrically determined by drying a 5 g of salad in a vacuum oven at 70 °C to constant weight [21]. Determinations were carried out in triplicate.

2.10. Statistical Analysis

The results were evaluated with the analysis of variance (ANOVA) method, and the significant differences were identified by the Tukey test at 5% significance using Statistica version 7.0.

3. RESULTS AND DISCUSSION

The level of phenolic compounds significantly differed ($p<0.05$) between non-irradiated (control) and irradiated samples – 0.5, 1.0, 2.0 and 3.0 kGy radiation doses led to a 4, 9, 9 and 14% drop in phytochemical content, respectively, when compared to control (Figure 1).

For flavonoids content, significant differences ($p<0.05$) between control and 1.0 and 3.0 kGy doses were observed (see Figure 1). Irradiation doses led to a rise of about 7% after delivered 1.0 kGy dose and to a drop of 12% in flavonoid level with 3.0 kGy dose. Samples irradiated with doses of 0.5 and 2.0 kGy did not significantly differed by control.

Significant differences ($p<0.05$) in proanthocyanidins content between control and irradiated samples were noted, shown in Figure 1. Losses of 5, 7 and 26% were associated with delivered doses of 0.5, 2.0 and 3.0 kGy, respectively. Proanthocyanidins were not significantly affected by 1.0 kGy dose when compared to control.

It is plausible that γ-irradiation may have degraded the phenolic content, once vegetables were submitted to a injury to produce minimally processed products. It can result in oxidative stress, which in turn can result in changes in the content and composition of the bioactive compounds [22]. Similar behavior in amount of total phenolic compounds was observed in previous studies conducted by De Toledo et al. [27] and Koseki et al. [24] in gamma-irradiated soybean grain and dehydrated rosemary (respectively). Losses about 20% were reported after delivered doses of 2 kGy in soybean, however, in rosemary the decreasing was observed after high irradiation doses (between 10 and 30 kGy).

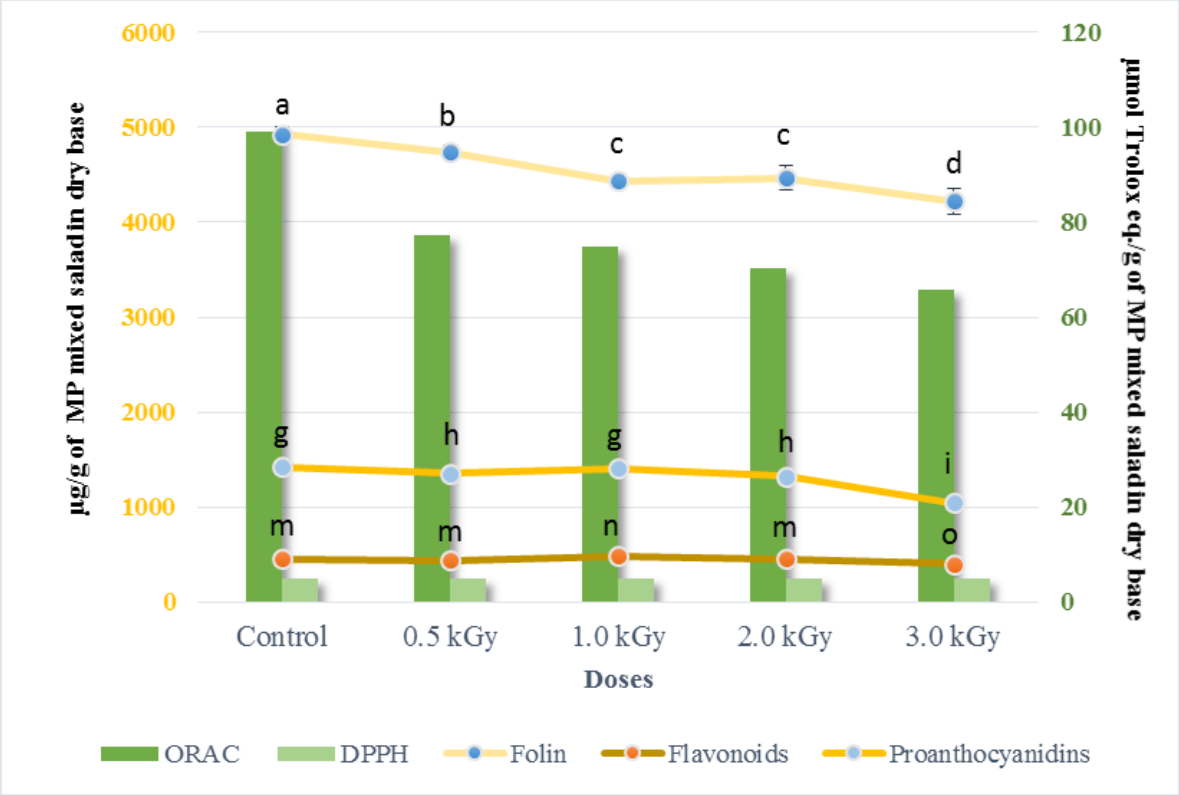

Figure 1: Levels of phenolic compounds, flavonoids, proanthocyanidins and antioxidant activity by DPPH• and ORAC assays of minimally processed mixed salad before and after irradiation. Bars and lines with standard deviation, expressed by error bars. Different letters indicate significant differences.

Secondary metabolites produced by plants are mainly found in the outer parts of fruit. Peeling and cutting could lead to the breakage of the protective glycosidic wall and consequent release of phenols. This way, they could be exposed and susceptible to the influence of treatments, such as irradiation, and may have their levels reduced, as observed in our study.

The method of radical 1,1-diphenyl-2-picrylhydrazyl (DPPH•) was chosen to evaluate the antioxidant activity of biactive compounds present in mixed salad due to be a simple and fast method with good and reproducible results. Meanwhile, no significant differences in antioxidant activity measured by DPPH• assay are found before and after irradiation.

By the ORAC method, a reduction in antioxidant capacity of 22% with a 0.5 kGy dose applied, 24% for 1.0 kGy, 30% for 2.0 kGy and 33% for 3.0 kGy were observed when compared to control. The reduction observed by this assay can induce a hypothesis that it could be more sensitive to the antioxidant activity of phenolic compounds, including flavonoids and proanthocyanidins, once a drop was observed in those compound levels after irradiation.

Others compounds with antioxidant capacity, not evaluated in this study, may have been extracted due to the solvent used. This fact may explain the maintenance of the activity measured by DPPH• assay, even after applying doses of ionizing irradiation, once these

substances may not have been damaged by the treatment. Several studies on plant materials showed that gamma irradiation may keep antioxidant properties [25-27]. Results reported by Murcia et al. [28] demonstrated no effect on antioxidant compounds and properties in spices, with irradiation up to 10 kGy.

Irradiation treatments have been also shown to either increase or decrease the antioxidant content of foods, which is dependent on phytochemical investigated, dose delivered, exposure time, and the raw material used [5, 27, 29-31].

4. CONCLUSIONS

In summary, phenolic, flavonoids, proanthocyanidins and antioxidant activity content present in minimally processed mixed salad significantly decreased with increasing irradiation dose. It is hypothesized that gamma irradiation, as a food processing method, may probably cause degradation or oxidation of the bioactive content. In spite of the reduction in the levels of bioactive compounds observed in this study, minimally processed mixed salad irradiated can keep nutritional benefits with low doses of irradiation. Further studies are being developed to confirm the results.

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