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Effect of gamma irradiation on antioxidants, microbiological properties and shelf life of pomegranate arils cv. 'Malas Saveh'

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ABSTRACT

In the present study, the effect of gamma irradiation on the reduction of microbial population and the maintenance of qualitative attributes of arils in pomegranate cv. Malas-e-Saveh in post-harvest stage was investigated. For this purpose, pomegranate arils, after being separated from the fruit, were irradiated with gamma doses of 0 (as control), 1, 3 and 5 kGy, packed in polyethylene container using cellophane film, and then maintained at 4 °C and relative humidity above 80%. After 7 and 14 days of storage, the arils were removed from the storage and were examined for count of bacteria, fungi and yeasts, anti-oxidant properties and other qualitative characteristics. The results indicated that gamma irradiation in all doses significantly reduced the population of bacteria, fungi and yeasts when compared with control. However, in comparison with control, samples irradiated with gamma irradiation, especially in high doses, contained lower soluble solids, titratable acidity, phenol, anthocyanin and ascorbic acid contents, antioxidant capacity and activity of polyphenol oxidase enzyme, but higher hydrogen peroxide content. Therefore the effects of gamma irradiation in low dose (1 kGy) on the quality of arils were not considerable and would be effective in increasing the shelf life of pomegranate arils.

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to Punicaceae family and is one of the oldest fruits widely cultivated in countries such as Iran, China, Spain and the United States. Pomegranate is native to Iran and northern India, but nowadays it is widely cultivated in sub-tropical regions of the world (Sarkhosh et al., 2009). The presence of various vitamins, minerals, fiber, polyphenols, anthocyanins, tannins and ellagic acid in pomegranate has caused the fruit to be highly considered at global level in terms of health aspects Pomegranate fruit juice contains 85% water and 10% sugar, mainly glucose and fructose (Mditshwa et al., 2013).

The edible portion of the pomegranate fruit is called aril that includes seeds, the outer part of which is fleshy and juicy. Aril forms 50% of the weight of pomegranate fruit (O'Grady et al., 2014). Extraction and separation of aril from pomegranate peel is a difficult and time-consuming process that can limit its consumption as a result of the rapid life of people (Kapetanakou et al., 2015). Therefore, the supply of pomegranate fruit as ready-to-use aril is of great economic importance. The supply of fruits, especially those with a large size or hard skin, as fresh-cut in diets is very useful (Zambrano-Zaragoza et al., 2014), but maintaining the edible quality and preventing the spread of microbes in fresh-cut crops are one of the most important challenges (O'Grady et al., 2014). In pomegranate aril, microbial contamination and rapid decline in quality, including loss of nutrients due to metabolic processes such as increased respiration or oxidation of phenolic compounds, are major factors limiting post-harvest life (Kapetanakou et al., 2015). Therefore, treatments that minimize these constraints should be used. There are some reports on the effects of several treatments on the increase of pomegranate aril shelf life such as: active modified atmosphere packaging (Banda et al., 2015), passive modified atmosphere packaging (Caleb et al., 2013), low and super atmospheric oxygen (Belay et al., 2017), edible coatings such as chitosan (Özdemir and Gökmen, 2017), carboxymethyl cellulose (Saba and Amini, 2017), Aloe Vera gel (Martínez-Romero et al., 2013) and nano-ZnO treatment (Saba and Amini, 2017). There is no report regarding the effect of gamma treatment on pomegranate aril.

Gamma irradiation is a physical treatment used as an alternative method to chemical treatments for eliminating all types of germs and insects, preventing germination of tubers and extending the shelf life of

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various types of fruits and vegetables in agriculture (Khan et al., 2012). ^{60}Cobalt and ^{137}Cesium radioisotopes are the approved sources of gamma irradiation for irradiating foods, but 60Cobalt, which is produced by neutronic bombardment of ^{59}Cobalt in nuclear reactors, is the main radioisotope used in irradiation of foods (Kim et al., 2009). Recently, gamma irradiation has been widely considered in post-harvest physiology and technology. This high-potential technique is used to inactivate all kinds of microorganisms and to maintain the qualitative properties of fresh fruits, and usually does not have adverse effects on fruits in low doses (Fan and Sokorai, 2008). For example, it has been shown in apple fruit that gamma irradiation, especially in doses of 300 and 600 Gy, was an effective treatment in maintaining the quality of apple stored at cold temperatures by prevention of disease incidence and better maintenance of firmness (Mostafavi et al., 2013). Shahbaz et al. (2014) investigated the effects of gamma irradiation in doses of 0, 0.4, 1 and 2 kGy on the quality of whole pomegranate fruit and showed that gamma irradiation reduced the amount of titratable acidity, phenol content, anthocyanin and antioxidant capacity of the fruit compared to the control samples. However, panel tester preferred the treated samples to control ones, due to the reduction of organic acid and sour taste of the fruits. The positive effect of gamma irradiation was also reported on the prevention of post-harvest decay of strawberry fruit (Alexandre et al., 2012) and the increase of antioxidant properties of raspberry fruit (Verde et al., 2013).

Given that microbial agents attack is the main factor limiting pomegranate arils shelf life and gamma irradiation is a safe treatment for reduction of microbial load in food, the effect of gamma irradiation treatment on the increase of post-harvest life of pomegranate aril, by reducing the microbial load, and on the antioxidant properties of pomegranate aril was explored.

2. Materials and methods

2.1. Fruit samples and treatments application

Fruits of pomegranate cv. Malas-e-Saveh, as the main exporting Iranian cultivar, were harvested at maturity stage from a commercial orchard located in Saveh city, Iran, and were transferred immediately to the laboratory of Agriculture and Medicine Nuclear Research center, Atomic Energy organization of Iran. After being washed with water, the fruits were cut and hand seeded and the damaged and inappropriate arils were discarded. Then, a sample containing 300 g aril, as 3 replicates, was analyzed for traits at the harvest time. The remaining arils were randomly divided into 24 polyethylene containers, each containing around 100 g aril, and were then exposed to 0 (control), 1, 3 and 5 kGy of gamma irradiation (6 containers for each dose) using a Gamma beam YR-530 nm irradiator having 60Co as a gamma irradiation source.

The irradiated arils were packed using cellophane film and stored at 4 °C and relative humidity over 80%. After 7 and 14 days of storage (DS), three packages of each treatment, as three replicates, were removed and evaluated for quantitative and qualitative traits (Ghasemnezhad et al., 2013).

2.2. Measurement of quantitative and qualitative traits

In order to measure total phenol content and antioxidant capacity, 5 g aril was first homogenized in 15 ml of 80% methanol, then obtained homogenate was centrifuged at 10,000 RPM at 4 °C for 15 min, and finally the supernatant was collected. To measure total phenol content, Folin Ciocalteu method was used (Sayyari et al., 2011). Antioxidant properties of aril were also determined by neutralization of DPPH free radical as described by Sayyari et al. (2011).

Ascorbic acid content was determined by 2,6-dichlorophenol indophenol (DCPIP) titration method (Tezotto-Uliana et al., 2013). An amount of 5 g aril was extracted by using 15 ml of 3% metaphosphoric acid, and the obtained extract was centrifuged at 10,000 RPM at 4 °C for 10 min. The supernatant was titrated against DCPIP dye until the appearance of faint pink color. The ascorbic acid content was expressed as mg/100 g fresh aril weight.

Total anthocyanins content in juice samples was determined by pH differential method using two buffer systems as described by Hmid et al. (2013). Briefly, 1 ml of diluted aril juice was mixed with 4 ml of potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M) in a separate test tube and the absorbance of solutions was measured at two wavelengths, 510 nm and 700 nm, by spectrophotometer against distilled water as a blank. Total anthocyanins content was calculated by using the formula of:

$$(\mathrm{mg/L}) = \frac{[(\mathrm{ABS\,510nm} - \mathrm{ABS700nm})\mathrm{pH\,1} - (\mathrm{ABS\,510nm} - \mathrm{ABS700nm})\mathrm{pH\,4.5}] \times \mathrm{MW} \times \mathrm{DF}}{\mathrm{MA}} \times 100$$

Where ABS: absorbance; MW: molecular weight (449.2 g/mol); DF: dilution factor (10); MA: molar absorptivity coefficient of cyanidin-3-glucoside (26.900).

A part of the arils of each experimental unit were extracted and soluble solids were measured using a manual refractometer (ATC-1e model). In order to measure the titratable acidity, 10 ml of the extract was mixed with 90 ml of distilled water and then obtained extract was titrated with 0.1% NaOH until reaching the final pH of 8.2, and titratable acidity percentage was calculated based on the dominance of citric acid (Hmid et al., 2013).

2.3. Microbial analysis

Microbial analysis of the arils was performed only at 14 DS. For measuring microbial growth of moulds and yeasts, 5 g aril was homogenized with 100 ml tryptone phosphate buffer by using a stirrer for 1 min. Then, one to ten dilutions were performed using saline peptone solution for ten times. For growth of bacterium, 8% Nutrient Agar (w/v) was used, and for growth of moulds and yeasts, potato dextrose agar was used at concentration of 8% along with 100 mg Oxytetracycline (for eliminating bacteria). An amount of 5 ml of the prepared culture media was poured in Petri dish, and after solidification, microbial suspensions were spread on them. The bacteria samples were incubated at 30 °C for 48 h and yeasts and moulds ones were incubated at 22 °C for 48 and 72 h, respectively, and then the number of colonies formed was counted and aril microbial count was expressed based on 10logCFU/g (Artés-Hernández et al., 2010).

2.4. Polyphenol oxidase enzyme activity

To measure the activity of polyphenol oxidase enzyme, 5 g aril was homogenized with 15 ml of 50 mM potassium phosphate buffer (pH 6.8) and centrifuged at 10,000 RPM at 20 °C for 20 min. The supernatant was collected and then 700 µl of it was mixed with 1800 µl of 50 mM potassium phosphate buffer and 500 µl of pyrocatechol solution (0.4 M). The absorbance changes of the resulting solution were recorded at 420 nm for 2 min and one unit of enzyme activity was defined as the amount of enzyme that caused an increase in OD_{420nm} per g fresh weight of sample per min (Gonzalez et al., 1999).

2.5. Hydrogen peroxide (H_2O_2) content

In order to measure the content of hydrogen peroxide, 2 g aril was homogenized with 10 ml of 0.1% trichloroacetic acid solution and then centrifuged at 10,000 RPM at temperature of 4 °C for 15 min. The supernatant was collected, and then 0.75 ml of it was mixed with 0.75 of 10 mM phosphate buffer (pH 7) and 1.5 ml of 1 M potassium iodide solution. The absorbance of the resulting solution was measured at the wavelength of 390 nm and the amount of hydrogen peroxide was calculated using standard diagrams prepared from different concentrations