

Evaluation of potential of gamma radiation as a conservation treatment for blackberry fruits

M. Oliveira^{a,b}, J. Pereira^b, S. Cabo Verde^{b,*}, M.G. Lima^a, P. Pinto^{a,c}, P.B. de Oliveira^d, C. Junqueira^b, H. Marcos^b, T. Silva^b, R. Melo^b, C.N. Santos^{c,e} and M.L. Botelho^b

^a*Escola Superior Agrária de Santarém – Instituto Politécnico de Santarém, São Pedro, Santarém, Portugal*

^b*Campus Tecnológico e Nuclear, Instituto Superior Técnico, Sacavém, Portugal*

^c*Instituto de Tecnologia Química e Biológica – Universidade Nova de Lisboa, Estação Agronómica Nacional, Oeiras, Portugal*

^d*Instituto Nacional de Recursos Biológicos, Unidade de Sistemas Agrários, Oeiras, Portugal*

^e*Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

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Abstract.

BACKGROUND: Blackberries consumption has been associated with health benefits. However, these fruits present a short shelf-life. Thus, food irradiation is a potential alternative technology for conservation of these fruits without use of chemicals.

OBJECTIVE: Analyse the potentiality of gamma radiation as a decontamination method for blackberry fruits.

METHODS: Fresh packed blackberries were irradiated in a Co-60 source at two doses (1.0 and 1.5 kGy). Bioburden, physical and rheological, sensorial and total soluble content parameters were assessed before irradiation, immediately after and at two days storage time at 4°C.

RESULTS: The characterization of blackberries microbiota point out to an average bioburden value of 10⁴ CFU/g and to a microbial population predominantly composed by filamentous fungi. The inactivation studies on the blackberries mesophilic population indicated a limited microbial inactivation (<1 log decimal reduction) for the applied radiation doses, being the surviving population mainly constituted by filamentous fungi and yeast. No effect of irradiation on colour of blackberries was observed. Concerning texture parameters, no significant differences were observed in both fracturability and firmness between non-irradiated and irradiated blackberries immediately after irradiation. In blackberries stored for two days, both parameters were slightly lower in irradiated blackberries, compared to non-irradiated blackberries. The performed sensorial analysis indicated a similar acceptability among irradiated and non-irradiated fruits.

CONCLUSION: This work reveals gamma irradiation treatment potential since no major impact was detected on blackberries physical, rheological and sensory attributes. Further studies with longer periods of storage are needed to elucidate the advantages of irradiation as a conservation treatment.

Keywords: Blackberries, food irradiation, food microbiology, rheological properties

1. Introduction

Vegetables and fruits provide most of the micronutrients of the human diet. They are also important sources of dietary fibre and phytochemicals. High consumption of fruits and vegetables has since long been associated with a lower risk of non-communicable chronic and inflammatory diseases, such as cardiovascular diseases, diabetes and

*Corresponding author: Sandra Cabo Verde, Campus Tecnológico e Nuclear, Instituto Superior Técnico, Estrada Nacional 10, 2686-953 Sacavém, Portugal. Tel.: +351 219946253; Fax: +351 219946285; E-mail: sandracv@ctn.ist.utl.pt.

some types of cancer [1, 2]. A large number of studies have been linking berry fruits consumption to a profound and positive effect on human health, due to its high content in polyphenols [3, 4]. Blackberries are characterized by a high content of anthocyanins and ellagitannins, as well as some flavonols and flavanols [5]. As reported by numerous studies, these polyphenols have shown anti-inflammatory, anticancer and neuroprotective properties, thus preventing cardiovascular and neurodegenerative diseases, diabetes and cancer [5–7].

A wide variety of fungi (mostly moulds) is capable of growing and spoiling various types of berries; considering the fact that these commodities contain high levels of sugar and other nutrients and a water activity ideal for fungal growth. Additionally, the low pH of these fruits eliminates the competition from many bacterial species, making it easier for fungi to grow and spoil the fruits. Fungal spoilage of fruits will depend on cultivation, harvesting, handling, transport, and post-harvest storage and marketing conditions. Post-harvest fruit spoilage results in significant economic losses [8]. Additionally, if the spoiling fungi are toxigenic or pathogenic, they could pose a health risk for the consumer. Some of these moulds could produce mycotoxins while grown on fruits even during refrigeration [9].

Based on that, blackberries are highly perishable fruits, with an usual shelf life of 2 to 3 days at refrigerated temperatures [10], showing great limitations regarding fresh market utilization. Therefore, it is of the most importance the utilization of techniques that can improve shelf-life time without altering physical, sensorial and nutritional characteristics of the food. Recent interests in these technologies are not only to obtain high-quality food with “fresh-like” characteristics, but also to provide food with improved functionalities. In addition to their possible beneficial effects on nutritional and bioactive content many of these novel technologies are more cost-efficient and environment friendly for obtaining premium quality foods which have led to their revival and commercialization [11].

In response to technological progress in manufacturing there are emerging alternative technologies in food conservation without use of chemicals, such as food irradiation. Irradiation is a “cold process” that, unlike thermic treatment can be used for pasteurization and sterilization of foods without causing modifications [12]. This treatment leave no toxic residues that are harmful to food showing more effectiveness in the reduction of the number of pathogenic microorganisms and/or deterioration and retards and/or eliminates natural biological processes, such as maturation, germination or microbial growth in fresh food [13–15].

There are three types of allowed radiation for food processing: gamma rays, X rays and electrons. Gamma rays are more utilized for food irradiation, being, at the moment, cobalt-60 the most chosen isotope for food irradiation because of its highly developed production, fabrication and encapsulation technology [14]. To provide a general indication of commercial processing costs (e.g. throughput more than 60 000 t/a), for a treatment dose up to 1 kGy the cost would be US \$1–3 per ton. The cost of irradiation may be discounted by the cost of the alternative process which irradiation replaces. An additional offsetting saving may be realized in treatments which prolong shelf-life or reduce waste, thus increasing the volume of saleable product and profits [16].

The aim of this study was to evaluate the potential of gamma radiation decontamination treatment for blackberries. The effect on microbial population was assessed after two days of storage. Food quality parameters such as colour, texture, total soluble solids and sensory characteristics were also evaluated.

2. Material and methods

2.1. Sampling and irradiation

Blackberries (*Rubus fruticosus*, cv. Primark) were grown in Fataca experimental field (Odemira, Portugal). Fully ripened fruits (1000 g) were harvested and packed in polystyrene boxes (125 g/package) with a lid and holes for air circulation.

Irradiations were carried out at the Co-60 experimental equipment (Precisa 22, Graviner, Lda, UK) in the Ionizing Radiation Unit located in the Campus Tecnológico e Nuclear, Sacavém, Portugal. Dosimetric studies were performed using an ionization chamber (FC65-P) to establish the irradiation geometry and estimate the dose rate (2.5 kGy/h). The obtained dose uniformity was 1.23. Packages of blackberries were irradiated at two doses (1.0 kGy and 1.5 kGy) in one irradiation batch (3 packages of 125 grams each/dose). Three packages of 125 grams each were not submitted to irradiation (non-irradiated samples). After irradiation, irradiated and non-irradiated samples were stored at 4°C

(regular retail and consumers home storage temperature) until analysis. Absorbed doses were monitored by routine dosimeters (Amber Perspex Harwell®, Batch V).

Microbiological, physical, rheological and sensory parameters were assessed in non-irradiated samples, immediately after irradiation (0 days of storage) and after 2 days of storage. Since usual blackberries shelf life is 2 to 3 days at refrigerated temperatures, we decided to test decontamination after 2 days storage at a regular retail and consumers home storage temperature of 4°C, as an assessment for gamma irradiation efficiency.

For each analysis, samples were prepared with fruits randomly collected from the three 125 g packages used in each of the different test conditions (non-irradiated, irradiated with 1.0 and 1.5 kGy). Microbiological analyses were performed on samples of 25 g; color and texture analyses were performed on samples of 10 fruits; total soluble solids were performed on homogenized juices of 5 fruits.

2.2. Microbiological inactivation studies

Samples of 25 g were blended on 100 mL of physiological solution with 0.1% of Tween 80 and homogenised in a stomacher equipment (Stomacher 3500; Seaward, UK). Serial decimal dilutions were performed before plating. Aerobic mesophilic counts were carried out, in triplicate, on *Tryptic Soy Agar* (Merk, Germany) at 30°C during 7 days. Microbiological counts were expressed as mean log colony forming units (CFU/g).

All colonies (microbial population from non-irradiated and irradiated blackberries) were macroscopically (e.g.: pigmentation, texture, shape), microscopically and biochemically typed by gram staining, catalase activity and oxidase test. The isolates were organized into typing groups according with Bergey's Manual of Determinative Bacteriology [17]. The frequency of each phenotype was calculated based on the number of isolates ($n = 428$ from samples with 0 days of storage; $n = 166$ from samples with 2 days of storage) and their characterization.

2.3. Colour assessment

Colour of blackberries was assessed with a Colorimeter KONICA MINOLTA CR 400. Ten measurements were performed for each irradiated and non-irradiated samples and data was analysed using *Spectra Magic Nx Software*. From the obtained values (L^* , a^* and b^*) the Chroma (C^*) and Hue (H°) values were determined. The L^* value represents the luminosity of the observed colour, measures the variation of the brightness between black (0) and white (100); the values of a^* and b^* are the chromaticity coordinates, where a^* sets the colour red (positive values) and green (negative values) and b^* sets the color yellow (positive values) and blue (negative values). The parameter C^* corresponds to purity, giving us a value farthest from the origin according to stronger and brighter color. H^* measure tonality and is represented by an angle from 0° to 360°. Angles between 0° and 90° represent the reds, oranges and yellows; 90° to 180° are yellow, yellow-green and green; 180° to 270° are green, cyans (blue - green) and blue; 270° to 360° are blues, purples, magentas and reds again.

2.4. Total soluble solids content

The total soluble solids (TSS) content was assessed with a refractometer ABBÉ. The equipment was previously calibrated at 24°C, with distilled water. Four measurements were done per sample and results were expressed as °Brix.

2.5. Texture

Textural attributes of blackberries were analysed with a *Stevens QTS-25* texturometer, using the puncture test (*Magness Taylor*) according to Table 1. Ten measurements were performed for each irradiated and non-irradiated samples. The evaluated textural attributes were: 1) fracturability (necessary force for breaking the material); 2) firmness (necessary force for bite penetration); 3) apparent modulus (rigidity of the food); 4) adhesive force (maximum force of attraction of the product). The results are reported as force in Newton (N).

Table 1
Test conditions for determination of the blackberries texture

<i>Test Condition</i>	
Type of assay	Penetration puncture test (needle)
“Trigger point”	1g
Target test	Distance
Velocity	80 mm/min
Number of cycles	1
Target Value	8 mm
Number of replicates	10 replicates/ dose

2.6. Sensory analysis

An untrained test panel consisting of five randomly select individuals (25 < age < 50; 40% smokers, 100% healthy subjects) was performed to assess the sensory quality of samples and factors determining refusal or acceptability of the product by the consumer. Evaluated parameters were: 1) exterior and interior colour, 2) smell, 3) taste, 4) fracturability, 5) firmness, 6) sweetness, 7) elasticity and 8) overall assessment. A hedonic scale was used, ranging from 1 (dislike extremely) to 5 (like extremely).

2.7. Data analysis

Statistical analyses were performed using the software *Statistica 10*. Data were subjected to analysis of variance (ANOVA) and significant differences among the means were determined by *Fisher Post hoc* test at a $P < 0.05$ significant level.

3. Results and discussion

3.1. Microbiological inactivation studies

The microbiological results indicated that blackberries presented an initial bioburden of 7×10^4 CFU/g. In order to analyse the response of blackberries microbial population to gamma radiation a survival plot was constructed based on the logarithmical values of the number of survivors in function of the absorbed dose. The obtained results do not show an inactivation tendency for the applied gamma radiation doses. However, for blackberries irradiated at 1 kGy it was observed a significant 0.9 log reduction of the number of survivors (86% inactivation efficiency) immediately after irradiation. Although, after two days of storage, it was verified an increase of the number of microorganisms present in 1 kGy irradiated fruit, that was not significantly different from the total counts obtained for 2 days storage non-irradiated blackberries. On the other hand, for 1.5 kGy the microbial inactivation efficiencies obtained were low, approximately 39% (reduction of 0.21 log) and 57% (reduction of 0.37 log) for blackberries immediately after irradiation and after two days of storage, respectively. These results could be related to the heterogeneity of blackberries microbiota.

Previous studies of gamma radiation effects on berries pointed out to limited microbial inactivation. For blueberry it was reported that inactivation of microbial load after irradiation, at doses between 0 up to 3 kGy (with intervals of 0.5 kGy) was reduced by approximately 1.5 log [18]. Also, in raspberries, a reduction of 1 log in the microbial population was observed after irradiation at 1.5 kGy [19].

The microbiota from non-irradiated and irradiated fruit were phenotypically characterized to evaluate the dynamics of blackberries microbial community and its pattern with radiation doses (Table 2).

The initial microbial population of non-irradiated fruits was mainly constituted by filamentous fungi, with the appearance of yeast during storage. A research on mould and yeast flora in fresh berries reported that 100% of black-

Table 2

Frequency of the morphological phenotypes of the isolates from non-irradiated and irradiated blackberries with storage time ($n = 428$ isolates from samples immediately after irradiation; $n = 166$ isolates from samples with 2 days storage after irradiation)

	% of total microbiota		
	Non irradiated	Dose (kGy)	
Phenotypical typification		1.0	1.5
<i>Immediately after irradiation</i>			
Gram negative oxidase negative rods	0.00	0.00	0.00
Yeast	0.00	15.49	60.32
Filamentous fungi	100.00	84.51	39.68
<i>2 days storage after irradiation</i>			
Gram negative oxidase negative rods	0.00	0.00	3.23
Yeast	20.00	32.35	41.94
Filamentous fungi	80.00	67.65	54.83

berry and raspberry, 97% of strawberry and 95% of blueberry samples showed some sort of fungal contamination [8]. According to these authors blackberry, raspberry and blueberry have significantly thinner, more susceptible to injury and breakage epidermis with numerous indentations and fiber-like protuberances where the various microorganisms can easily attach and invade the inner tissues of the fruits. The same study mentioned a lower yeast incidence in berries that was partially explained by the fact that these organisms cannot break the fruit epidermis and infect the inner fruit tissues. Considering this explanation, the appearance of yeast after storage could be related to a slight softening of fruit epidermis, although this effect was not verified in the analysed texture parameters (see section 3.4).

Also after irradiation, either immediately after irradiation or after two days storage, it was noticed the presence of yeast that augmented the frequency with the increase of the radiation dose. These different microbial patterns can be explained considering the inactivation of the more sensitive microorganisms with increasing dose, thus increasing the frequency of the most resistant.

The surviving microbiota of irradiated blackberries seems to be homogenous along storage time, with the prevalence of filamentous fungi and yeast. Other study also reported that yeast and mold populations dominated the microflora of fresh-cut pineapples and strawberries under MAP (Modified Atmosphere Packaging) and refrigerated storage (7°C) [20]. On raspberries microbiota, filamentous fungi was also the predominant morphotype after irradiation at 1.5 kGy [19].

Pathogenic fungi in berries that cause most foodborne diseases have been reported to be *Penicillium*, *Botrytis*, *Colletotrichum*, *Mucor* and *Monilinia* [20]. For the majority of fungi the sufficient mean lethal dose is 5 to 10 kGy. The observed increase in yeast frequency with the irradiation dose may be explained by the higher resistance of yeast to irradiation doses than moulds and vegetative bacteria [21]. The same results were reported in cherries treated with gamma radiation [22].

3.2. Colour assessment

Surface colour is one of the most appealing factors that influence the consumer in the purchase of fruit. Therefore evaluation of the global colour properties was done for irradiated and non-irradiated blackberry fruits (Table 3). No significant differences were observed in lightness and chroma values between non-irradiated and irradiated blackberries, either immediately after irradiation or after two days storage (Table 3). Tonality (Hue value) was lower in blackberries immediately after irradiation, indicating a higher tendency for red, compared to non-irradiated samples. However, these results were not observed between samples stored for two days, where no significant differences were found between non-irradiated and irradiated samples. A decrease in hue values with storage was also observed. According to other authors the hue angle decreases in storage due to anthocyanin synthesis, responsible for the red colour on berry fruits [23]. A previous study reported that irradiation at doses between 0.5 and 1.5 kGy did not have

Table 3

Colour parameters for non-irradiated and irradiated blackberry fruits. Mean values \pm SD (standard deviation) obtained for the colorimetric parameters for non-irradiated and irradiated samples ($n = 10$)

Sample	L* \pm SD	C* \pm SD	H° \pm SD
<i>Immediately after irradiation</i>			
Non irradiated	18.91 ^{abd} \pm 1.00	1.23 ^{ab} \pm 0.36	36.45 ^d \pm 15.30
1.0 kGy	20.06 ^{ab} \pm 1.31	1.20 ^a \pm 0.27	22.89 ^b \pm 6.87
1.5 kGy	19.76 ^{ab} \pm 1.10	1.25 ^{ab} \pm 0.36	19.24 ^{ab} \pm 8.65
<i>2 days storage after irradiation</i>			
Non irradiated	17.47 ^{cd} \pm 1.05	1.68 ^b \pm 0.35	15.10 ^{abc} \pm 5.75
1.0 kGy	16.79 ^c \pm 0.63	1.46 ^{ab} \pm 0.51	7.86 ^c \pm 2.13
1.5 kGy	18.57 ^{acd} \pm 1.17	1.55 ^{ab} \pm 0.44	10.71 ^{ac} \pm 6.51

L*- lightness; C*- chroma; H° - hue. For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ($p > 0.05$).

Table 4

Total soluble solids (TSS) of non-irradiated and irradiated blackberry fruits ($n = 4$)

TSS (° Brix) at 24°C (Mean \pm SD)	
<i>Immediately after irradiation</i>	
Non irradiated	9.56 ^d \pm 0.43
1.0 kGy	9.06 ^b \pm 0.24
1.5 kGy	8.75 ^{ab} \pm 0.29
<i>2 days storage after irradiation</i>	
Non irradiated	9.25 ^{cd} \pm 0.35
1.0 kGy	10.00 ^c \pm 0.00
1.5 kGy	8.63 ^a \pm 0.32

The values between treatments that have the same letters are not considered significantly different ($p > 0.05$).

a pronounced effect on the colour of raspberries. However, the authors also found a decrease in colour parameters after two days storage in both irradiated and non-irradiated fruit [19]. A study with strawberries, reported that gamma irradiation increased the rate of change of fruit color from green to red [24].

3.3. Total soluble solids

Other important fruit quality parameter is TSS content. Mean values of TSS for irradiated and non-irradiated blackberries analysed immediately after irradiation and two days after storage at 4°C are shown in Table 4. The mean values of TSS of non-irradiated samples are slightly higher than those found in the literature ($\approx 8.0^\circ$ Brix) [25]. Results show that TSS decreased immediately after irradiation, and were dependent on the irradiation dose (Table 4). After two days of storage no significant changes occur in this parameter for non-irradiated blackberry fruits or after 1.5 kGy irradiation. Only an increase was found for samples irradiated with 1.0 kGy. According to other authors the TSS of blackberries do not change during storage, suggesting that sugar gain as a result of fruit ripening was balanced with respired sugar [26]. A study in kiwifruits with low-dose electron beam (e-beam) irradiation (0, 0.3 and 0.6 kGy) indicated that the soluble solids contents in irradiated kiwifruit were higher than non-irradiated fruit for the initial storage period; however a lower increase rate was verified during the storage period [27]. For seeds of anise exposed to gamma radiation doses of 0, 5, 10 and 20 kGy it was observed that immediately after irradiation, the TSS content of irradiated seeds were higher than those of non-irradiated [28].

Table 5
Texture parameters for non-irradiated and irradiated blackberry fruits. Average values \pm SD for texture parameters of the samples in study ($n = 10$)

	Fracturability \pm SD	Firmness \pm SD	Rigidity \pm SD	Adhesive Force \pm SD
<i>Immediately after irradiation</i>				
Non irradiated	0.09 ^{ab} \pm 0.08	0.13 ^a \pm 0.15	0.80 ^a \pm 0.44	0.05 ^{ab} \pm 0.04
1.0 kGy	0.09 ^{ab} \pm 0.05	0.22 ^a \pm 0.10	0.58 ^a \pm 0.63	0.16 ^c \pm 0.06
1.5 kGy	0.08 ^{ab} \pm 0.12	0.15 ^a \pm 0.12	0.20 ^a \pm 0.37	0.08 ^b \pm 0.07
<i>2 days storage after irradiation</i>				
Non irradiated	0.22 ^b \pm 0.15	0.41 ^b \pm 0.18	0.33 ^a \pm 0.17	0.03 ^{ab} \pm 0.02
1.0 kGy	0.07 ^a \pm 0.05	0.17 ^a \pm 0.05	0.44 ^a \pm 0.37	0.02 ^a \pm 0.02
1.5 kGy	0.07 ^a \pm 0.03	0.21 ^a \pm 0.11	0.65 ^a \pm 0.50	0.03 ^{ab} \pm 0.02

The results are reported as force in Newton (N). For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ($p > 0.05$).

3.4. Texture

Texture is another factor critical to consumer acceptance of a product. The term “firmness of fruit” is commonly used to describe a parameter assessed by means of empirical mechanical tests and understood as an attribute that ought to be maintained during storage and processing. Firmness, interpreted as a mechanical response intrinsic to the fruit structure, is influenced by the stage of physiological development, degree of ripeness, damage and identification, fibrousness and turgidity [29].

Mean values of texture parameters for the different blackberries samples are shown in Table 5. No significant differences were observed in both fracturability and firmness between non-irradiated and irradiated blackberries immediately after irradiation. In blackberries stored for two days, both parameters were slightly lower in irradiated blackberries, compared to non-irradiated blackberries.

On apples, a decrease of firmness was observed immediately after irradiation with doses from 0.5 to 1.5 kGy. The firmness decrease was higher with higher doses [24]. The same results were obtained for fresh strawberries irradiated with e-beam at 0, 1 and 2 kGy [30]. A loss of firmness was also observed in apricots and peaches for irradiation doses higher than 0.6 kGy [15]. Moreover, it was observed a decrease in texture in irradiated raspberries, however, the firmness of the raspberries treated with radiation doses of 1.0 and 1.5 kGy was not severely affected during the storage period [19]. Regarding rigidity no significant differences were detected between non-irradiated and irradiated blackberries (Table 5), either immediately after irradiation or after two days storage. Adhesive force was higher in blackberries immediately after irradiation with 1.0 kGy, than in non-irradiated samples. However, these results were not observed for samples stored for two days, where no significant differences were found between non-irradiated and irradiated samples.

3.5. Sensory analysis

The characterization of the sensory properties of blackberry fruits was analysed by the radar chart constructed with the ratings obtained for the different parameters evaluated by the panellists.

Flavour and sweetness were the parameters where the evaluation was most different. Blackberries irradiated at 1.5 kGy showed the highest punctuation in both parameters immediately after irradiation. However, after two days storage the samples rated as sweeter were the blackberries irradiated at 1.0 kGy, which is in agreement with Brix values. In a study with minimally processed fruits and vegetables, it was observed that irradiation did not influence watermelon sweetness or pineapple sourness [15]. Firmness and fracturability were evaluated by the panel with a lower rate for the irradiated samples with two days storage than the non-irradiated samples, showing agreement with the results obtained in texture analysis.

Immediately after irradiation there were no differences in the evaluation of fracturability in irradiated and non-irradiated samples. However, firmness was higher in irradiated than in non-irradiated samples.

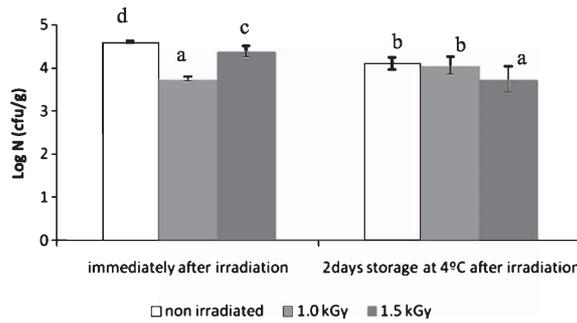


Fig. 1. Total microbial population survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries (1.0 kGy light gray bar and 1.5 kGy dark gray bar). Standard deviation bars correspond to 95% confidence intervals about mean values ($n = 3$; $\alpha = 0.05$). Bars with the same letters are not considered significantly different ($p > 0.05$).

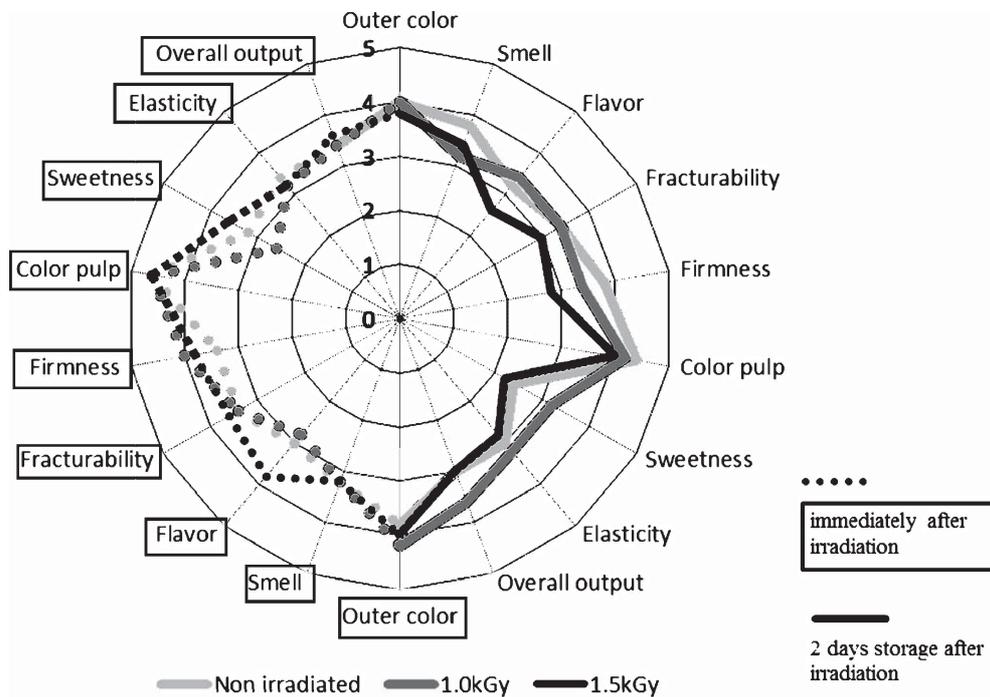


Fig. 2. Comparison of variation of the parameters studied in sensory analysis for blackberries irradiated to different doses and non-irradiated samples.

In the overall output, non-irradiated and irradiated blackberries were almost equally rated immediately after irradiation. Within two days storage, the rates lowered for non-irradiated and blackberries irradiated at 1.5 kGy, but not for blackberries irradiated with 1.0 kGy. In another study it was reported that blueberries exposed up to 1.6 kGy dose were found acceptable by the panelists in terms of overall quality, colour, texture, and aroma [18].

4. Conclusion

Summing up, it can be mentioned that an irradiation dose up to 1.5 kGy, does not result on a major impact on blackberries physical, rheological and sensory attributes. Nevertheless, low microbial inactivation efficiency was

verified for the applied gamma radiation doses. This study was important to redefine the methodological setup to assess the feasibility of irradiation as a conservation treatment for longer periods, therefore aiming to increase blackberry fruits shelf-life. As so, the effect of higher irradiation doses on fruit will be further investigated, in an attempt to augment the reduction of the microbial population of blackberries. As mentioned by other authors refrigerated storage affects the bioactive compounds and had inconsistent impacts on the antioxidant capacity of two blackberry varieties. These results suggested that other treatments along with refrigerated storage are necessary for prolonging shelf life and retaining nutraceutical benefits of fresh blackberries [26]. Taking this in consideration, the impact of irradiation on chemical and nutritional parameters of blackberries will also be studied. Phytochemicals stability during the process is also an important aspect to be considered in blackberry fruits. Overall this work reveal that gamma irradiation treatment have high potential to be further evaluated as a conservation treatment in blackberry fruit.

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