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Changes in the volatile compounds of pork loin (fresh and marinated) with different irradiation and packaging during storage

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RESUMEN

Cambios en los compuestos volátiles del lomo de cerdo (fresco y adobado) con diferentes irradiaciones y empaquetados durante el almacenamiento

Se ha utilizado la cromatografía de gases/espectrometría de masas, la extracción mediante purga y trampa para estudiar los compuestos volátiles de lomo de cerdo fresco y adobado, tratados con electrones acelerados (1 y 2 kGy) y almacenado en refrigeración (4 y 8 °C) bajo diferentes atmósferas (aire, vacío y atmósfera modificada). Se observaron diferencias importantes entre las muestras de lomo fresco y adobado pero, en general, solo pequeñas diferencias fueron observadas en algunos compuestos volátiles de ambos tipos de lomo debidas al efecto de la temperatura, tiempo de almacenamiento, tipo de atmósfera o dosis de radiación. Se ha concluido que la aplicación de electrones acelerados es una tecnología muy eficaz para ampliar la vida útil del lomo de cerdo fresco y adobado sin que se detecten cambios en el olor de los productos.

PALABRAS CLAVE: Adobado – Compuestos volátiles – Empaquetado – Fresco – GC-Purga y Trampa – Lomo de cerdo – Radiación de haz de electrones.

SUMMARY

Changes in the volatile compounds of pork loin (fresh and marinated) with different irradiation and packaging during storage

The analysis of volatile compounds by gas chromatography-mass spectrometry after extraction by purge and trap has been used to investigate the volatile compounds of fresh and marinated pork loin after E-beam treatmet as a function of packaging type (air, vacuum and modified atmosphere), radiation dose (1 and 2 kGy) and storage temperature (4 and 8 °C). Major differences were found between fresh and marinated samples but, in general, only minor differences were found in the volatile compounds of both types of loin due to storage temperature, packaging method and doses of irradiation. It is concluded that the application of E-beam is a very useful way to extend the shelf-life of fresh and marinated pork loin with no changes in the odor of the products.

KEY-WORDS: E-beam irradiation – Fresh – GC-Purge and Trap – Marinated – Packaging – Pork loin – Volatile Compound.

1. INTRODUCTION

Worldwide, the population of pigs for human consumption rises to 956 million. The pork production contributes over 39% of the global production of meat for human consumption, an equivalent of 15.3 kg of pork consumed per person per year (MAPA, 2006). In Spain, the annual quantity per capita goes up to 58 kg of pork. To meet this demand, 37.5 million hogs are sent to slaughter annually. They are often killed when they turn 6 months old and weigh 100 kg. In the EU, this figure rises to 240 million pigs annually sent to slaughterhouses (MAPA, 2006).

The food industry has made great efforts to improve the maintenance of sanitary conditions and prevent the contamination of food, although a number of pathological processes associated with food still remain. The level of contamination can be reduced by good hygiene practices, but some pathogens are impossible to eliminate, especially in raw foods with minimal processing. Irradiation is presented as a possible method of decontamination for this food group. The most common alterations in the microorganisms in meat are Gram negative psychrotrophs which, in turn, are very susceptible to radiation because they are practically eliminated by a dose of 1 kGy (Monk *et al.,* 1995). Irradiation is also a very effective way to eliminate the pathogens present in foods, including L. monocytogenes (Patterson and Damoglou, 1993, Sommers *et al*., 2003, Zhu *et al*., 2005) and Salmonella spp (Grant and Patterson, 1991, 1992, ICMSF, 1996, Patterson 1988, Tarkowski *et al*., 1984, Thayer *et al*., 1990, Cabeza *et al*., 2009, Cabeza *et al*., 2007).

The quality of the meat may be affected, depending on dose, temperature, and atmosphere during treatment as well as storage conditions. As seen in various studies, the irradiation of meat can produce changes in its aroma, color and flavor, which can significantly affect consumer acceptance (Thayer, 1993, Ahn *et al*., 1998, Ahn, *et al*., 2000; Chouliara *et al*., 2006, Jo and Ahn, 2000, Samelis *et al*., 2005). In addition, these factors influence

oxidative chemical changes (Katusin-Razem *et al*., 1992).

An array of flavor- and odor-active volatiles occurs in meat (acids, alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons, ketones, lactones, pyrazines, pyridines, pyrroles, sulfides, thiazoles, thiophenes, pyrroles, and oxazoles (Shahidi, 1994, Lorenz *et al*., 1983). Several authors indicate that irradiated meat, regardless of packaging methods, produced more volatile compounds than non-irradiated meat and developed a distinctive smell after irradiation (Ahn *et al*., 1998). This characteristic odor has been described as metallic, sulfide, wet dog, wet grain, worse, rotten egg, sweet, bloody, cooked meat, barbecued corn, burnt, sulfur, metallic, alcohol, acetic acid, liver-like serumy, and bloody (Huber *et al*., 1953, Groninger *et al*., 1956, Hampson *et al*., 1996; Jo *et al*., 1999, Lee *et al*., 1996a, Lee *et al*., 1996b, Luchsinger *et al*., 1997c, Merritt *et al*., 1975). Some of the precursors of the off-odor compounds which are water-soluble contain nitrogen and/or sulfur (Schweigert *et al*., 1954).

One of the main defects of irradiated meat is this characteristic odor, which is produced by the oxidation of lipids in the presence of oxygen. In raw meat, odors can be developed or disappear during cooking (Luchsinger *et al*., 1996, Hashim *et al*.,1995 and Ahn *et al*., 1998). Most of the chemical changes in irradiated meat are associated with free radical reactions (Ahn and Lee, 2004). The characteristic odor of the irradiation process is supposed to be the result of oxidation of the free acids. Changes in the chemical oxidation by E-beam radiation depend on the dose and the presence of oxygen has a significant effect on the development of odor and its intensity (Merritt *et al*., 1975). Free radicals formed by this process interact with most organic molecules such as proteins, lipids, etc (Kim *et al*., 2008, Ahn 2001, Patterson and Stevenson, 1995) and they are clearly different from the characteristics of the oxidation of lipids.

Fatty acids are important precursors of the flavor of pork, because they are the main source of carbonyl compounds by heating (Selke *et al*., 1977, 1980). Therefore, carbonyl compounds are important for the odor of irradiation and its intensity depends on the essence of oxygen during irradiation (Reineccius, 1979).

The most important substance in the changes in meat quality are lipids, the effect of the fat content of irradiated meat is limited in the development of lipid oxidation, color changes or the production of volatiles production (Jo *et al*., 1999). A considerable amount of researches had been devoted to the study of the volatile compounds of meat. Among these studies, Ahn *et al*. (2001) researched the effect of irradiation on the volatile compounds of pork during storage, with different packaging. The volatiles were analyzed using the dynamic headspace GC / mass spectrometry method. Studying the gas chromatograms of irradiated raw pork suggested that the odor is caused by radiolytic protein degradation and lipid oxidation. Irradiation had a significant impact on pork in the number and profile of volatile compounds. Butane, propane, mercaptomethane, dimethyl sulfide, methyl thioacetate and dimethyl disulfide were produced by irradiation, and were not detected in non-irradiated pork. Kim *et al*., (2008) also showed that irradiated pork samples formed a greater number of volatile compounds and increased their contents. They were identified by SPME GC / MS. On the other hand Huang *et al.,* (2010) studied the contribution of the flavor of triglycerides and phospholipids of pork and observed a difference in taste between two breeds of pigs. The volatile compounds were extracted using solid phase microextraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). Once identified, they were grouped into classes of lipid-derived aldehydes, Maillard derived aldehydes, alkanes, ketones, alcohols, sulfur compounds containing nitrogen-containing compounds, and furans.

The aim of this study was to investigate the effect of electron-beam irradiation on the volatile compounds in raw and marinated pork loin with different packaging and storage times.

2. EXPERIMENTAL

2.1. Reagents and standards

2-butanone, Pentanal, Hexanal, 2-heptanone, Heptanal, 2-hexenal, 2-octanone, Octanal, 2-heptenal, 6-methyl-5-hepten-2-one, 2-nonanone, Nonanal, 2-octenal, Decanal, Nonenal, 2-decenal and Isoamyl butyrate were obtained from Sigma Aldrich Fluka (Steinheim, Germany). Standard solutions were prepared using fully deodorized edible oil as matrix. Concentrations were in the range of 0.1-5.0 μg g^{-1}.

2.2. Samples and Sample treatment

A total of fifty-four slices of fresh (Garcia-Marquez *et al*., 2012a) and marinated (Garcia-Marquez *et al*., 2012b) pork loin were packaged into low gas permeability laminated plastic bags (diffusion coefficient of 35 cm^3/24 h m^2 bar for O2 and 150 cm^3/24 h m^2 bar for CO2) with a 5:1 (v/w) gas/product ratio. Three batches were made. An aerobically packaged batch was used as control and the remainder were packaged in either a vacuum or a carbon dioxide enriched atmosphere (CO2/O2/N2) (30/20/50) (v/v/v) by means of a thermo forming packaging machine, model TMM 37/28 (Vapta, Madrid, Spain).

Samples were treated in an industrial electron beam radiation source working at the energy of 10 MeV. The radiation doses employed were 1 and 2 kGy. The dose absorbed by the samples was verified considering the absorbance of cellulose triacetate dosimeters (ASTM, 2000) simultaneously irradiated. Following the irradiation treatment, they were stored in termostated chambers at 4 and 8 °C,

the latter as an example of temperature abuse during product storage and distribution. Table 1 shows the E-beam treatment applied and the identification code assigned to each one.

2.3. Volatile compound analysis

Extraction of volatile compounds. The volatile compounds were isolated from 1.5 g of minced sample by the dynamic headspace technique and adsorbed on a Tenax trap, using a Purge and Trap (P&T) Concentrator apparatus Tekmar velocity XPT (Thousand Oaks, CA, USA), based on the method described by Narváez-Rivas *et al.,* (2010). The purge conditions were as follows: sample temperature, 45 °C; Tenax trap temperature, 35 °C; purge gas flow, 350 mL min^{-1} of nitrogen; purge time, 14 min. After the purge time, the volatile compounds were desorbed by heating in the Tenax trap at 225 °C for 1min, and sent through the transfer line (kept at 150 °C) into the chromatograph injector.

Gas chromatography/mass spectrometry (GC/MS) analysis. The GC-ion-trap-MS analyses were performed using a Varian 3800 gas chromatograph coupled to a Saturno 2000 ion trap mass spectrometer (Varian, Palo Alto, CA, USA). The system was equipped with a 1079 injector operating in full scan mode from 50 to 600 amu at 1 scan sec^{-1} for the purpose of identification . The column used was a Supelcowax-10 (SUPELCO, Bellefonte, PA, USA) fused silica capillary column (60 m long × 0.25 mm i.d. × 0.25 µm film thickness). The GC conditions included hydrogen as carrier gas at 1.6 mL min^{-1} in constant flow mode. The oven temperature was held at 40 °C for 14 min and then raised to 91 °C at 1 °C min^{-1}, and then to 201 °C at 10 °C min^{-1}, and then to 220 °C at 5 °C min^{-1}, where it was held for 20 min. Split injection mode was used with a ratio of 1:5. The injector temperature was kept at 250 °C. The MS operating conditions were the following: ion source and transfer line temperatures were 200 and 290 °C, respectively; the electron energy was 70 eV with a resolution of 1 and the emission current 250 µA; dwell time and inter-channel delay were 0.08 s and 0.02 s, respectively. For GC-ion trap-MS, Varian MS Workstation version 6.3 software was used for data acquisition and processing of the results. The aldehydes and ketones present in the volatile fraction of the fat samples were identified by computer matching of their mass spectra with those from NIST (National Institute of Standards and Technology) and Wiley libraries and verified by standards purchase from Sigma-Aldrich and Fluka (S. Louis, MO). Peak area was used as analytical signal.

2.4. Quantitative analysis and statistical treatment

Thirty-seven volatile compounds were identified. The peak areas of the volatile compounds were used as analytical signal. The quantification of individual volatile compounds was carried out using isoamyl butyrate as internal standard, which was prepared in refined sunflower oil (14.3407 mg 100 g^{-1} of oil). An equal relative response factor for any species was assumed. Isoamyl butyrate was used as a reference to calculate the relative retention time, due to the fact that it appears in all samples with high intensity at a mean retention time of 29.52 min. A representative chromatogram report of the volatile compounds of pork loin and their corresponding peaks are shown in Figures 1A and 1B. The relative retention time, molecular ion and base peak of the corresponding peaks are included in Table 2.

The volatile compounds identified were considered as chemical descriptors. A data matrix, whose rows are the samples and whose columns are the variables, was built. Each element of this matrix xij corresponds to the content of volatile compounds j for the sample i. Statistical analyses based on non-parametric techniques were used, including the Kolmogorov-Smirnov-Lilliefors test, which was used to evaluate the normality of each variable included in the study. Since the data distribution was not normal, non-parametric tests were applied. The Kruskal-Wallis test was used to find out significant differences among the variables with three levels. This test is considered as an ANOVA test for one factor. The Mann-Whitney U test was used to determinate the differences between two levels of a same variable. This test is considered similar to a t-Student test for independent samples groups. The calculations were made using the statistical package CSS: STATISTICA from StafsoftTM (Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Volatile identification

3.1.1. Purge and trap GC-MS analysis

A total of thirty seven volatile compounds were tentatively identified in the volatile fraction from pork loin (fresh and marinated) for the first time using P&T-GC-MS. A tentative assignment of the chromatographic peaks was done by comparing the spectra with those from NIST (National Institute of Standards and Technology) and WILEY libraries and verified by standards purchased from Sigma-Aldrich and Fluka (S. Louis, MO).

The volatile components of the samples were separated using a high polarity column and the conditions of the purge and trap system and GC-MS were previously described (Narváez-Rivas *et al*., 2010). Under the conditions used in the purge step no degradation of the matrix sample was observed. Repeatability was checked by consecutive analysis of one sample for 12 times and the values expressed as relative standard deviation ranged between 15.3 and 28.7%.

Table 1
Analysed intramuscular fat from pork loin samples

Code	Type	Temperature (°C)	Atmosphere	Radiation (kGy)	Time (days)
1F	Fresh	4	Air	0	0
2F	Fresh	4	Air	1	0
3F	Fresh	4	Air	2	0
4F	Fresh	4	Vacuum	0	0
5F	Fresh	4	Vacuum	1	0
6F	Fresh	4	Vacuum	2	0
7F	Fresh	4	MAP	0	0
8F	Fresh	4	MAP	1	0
9F	Fresh	4	MAP	2	0
10F	Fresh	4	Air	0	10
11F	Fresh	4	Air	1	10
12F	Fresh	4	Air	2	10
13F	Fresh	4	Vacuum	0	10
14F	Fresh	4	Vacuum	1	10
15F	Fresh	4	Vacuum	2	10
16F	Fresh	4	MAP	0	10
17F	Fresh	4	MAP	1	10
18F	Fresh	4	MAP	2	10
19F	Fresh	8	Air	0	10
20F	Fresh	8	Air	1	10
21F	Fresh	8	Air	2	10
22F	Fresh	8	Vacuum	0	10
23F	Fresh	8	Vacuum	1	10
24F	Fresh	8	Vacuum	2	10
25F	Fresh	8	MAP	0	10
26F	Fresh	8	MAP	1	10
27F	Fresh	8	MAP	2	10
1M	Marinated	4	Air	0	0
2M	Marinated	4	Air	1	0
3M	Marinated	4	Air	2	0
4M	Marinated	4	Vacuum	0	0
5M	Marinated	4	Vacuum	1	0
6M	Marinated	4	Vacuum	2	0
7M	Marinated	4	MAP	0	0
8M	Marinated	4	MAP	1	0
9M	Marinated	4	MAP	2	0
10M	Marinated	4	Air	0	10
11M	Marinated	4	Air	1	10
12M	Marinated	4	Air	2	10
13M	Marinated	4	Vacuum	0	10
14M	Marinated	4	Vacuum	1	10
15M	Marinated	4	Vacuum	2	10
16M	Marinated	4	MAP	0	10
17M	Marinated	4	MAP	1	10
18M	Marinated	4	MAP	2	10
19M	Marinated	8	Air	0	10
20M	Marinated	8	Air	1	10
21M	Marinated	8	Air	2	10
22M	Marinated	8	Vacuum	0	10
23M	Marinated	8	Vacuum	1	10
24M	Marinated	8	Vacuum	2	10
25M	Marinated	8	MAP	0	10
26M	Marinated	8	MAP	1	10
27M	Marinated	8	MAP	2	10

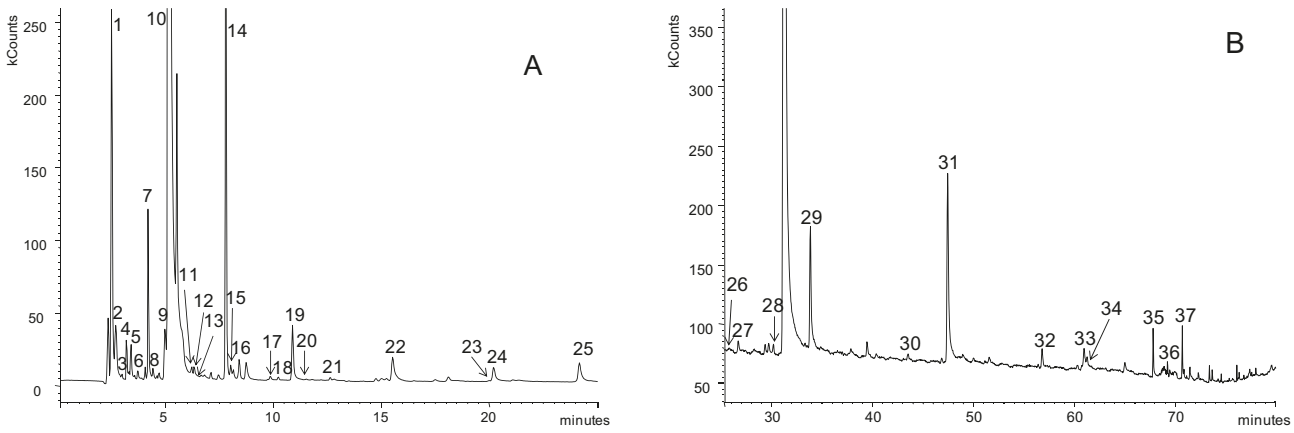

Figure 1
GC-ion-trap-MS chromatograms in full scan mode of total volatile compounds profile from pork loin: A, from 0.0 to 25.0 minutes; B, from 25.0 to 80.0 minutes. Peaks identification: see table 2.

Recoveries for the analytes with available standard varied between 95 and 119%. Figure 1 (A and B) shows a chromatogram of the volatile fraction of pork loin (fresh and marinated). The relative retention time, molecular ion and base peak of the corresponding peaks are included in Table 2. Several volatile compounds are present in this volatile fraction. A total of ten hydrocarbons were detected such as 2,4-dimethyl-hexane, 3-methyl-hexane, 2,5-dimethyl-hexane, 2,4-dimethy-heptane, 2-octene, 3,5,5-trimethyl-1-hexene, 2-beta-pinene, 3-carene, dl-limonene, and 2-pentyl-furane. Eight aldehydes such as: pentanal, hexanal, heptanal, 2-hexenal, octanal, nonanal, decanal, and 2-decenal. Eight alcohols: 2-(1-methylethoxy)-1-propanol, 2-butanol, 3-methyl-1-butanol, 1-hexanol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol. Five esters: 2-hydroxypropanoic acid ethyl ester, ethyl ester acetic acid, acetic acid ethenyl ester, ethyl ester butanoic acid and ethyl ester hexanoic acid. Four ketones: 2-propanone, 2-butanone, 2-pentanone, and 1-(methylphenyl)-ethanone have been detected, Along with other volatile compounds like chloroform, dimethyl disulphide and 2-nitrobutane.

Some of these compounds have been previously described in pork meat by several authors (Ahn *et al.*, 1998, Ahn, *et al.*, 2000; Ahn *et al.,* 2001; Jo and Ahn, 2000; Kim *et al.*, 2008). They are: dimethyl disulphide, pentanal, hexanal, heptanal, octanal, nonanal, decanal, hexanol, heptanol, 2-pentanona, 2-butanone, and 2-octene. Nonetheless, there are 25 other volatile compounds detected in the loin of pork that have been identified for the first time in this study such us: 2,4-dimethyl-hexane, 3-methyl-hexane, 2,5-dimethyl-hexane, 2,4-dimethy-heptane, 3,5,5-trimethyl-1-hexene, 2-beta-pinene, 3-carene, dl-limonene, and 2-pentyl-furane, 2-hexenal, 2-decenal, 2-(1-methylethoxy)-1-propanol, 2-butanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, 1-octanol, and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, 2-hydroxypropanoic acid ethyl ester, ethyl ester acetic acid, acetic acid ethenyl ester, ethyl ester butanoic acid, ethyl ester hexanoic acid, 2-propanone, 1-(methylphenyl)-ethanone, chloroform, and 2-nitrobutane.

3.2. Volatile compounds in fresh and marinated loin

Table 3 shows the median minimum and maximum values of the volatile compounds analyzed in the loin (as mg kg^{-1} of fat) corresponding to the fresh and marinated. In this table, it can be deduced several interesting observation. Firstly, it would be interesting stand out that there are five compounds which have been detected only in marinated samples, they are: 2-beta-pinene, 2-nitrobutane, 3-carene, dl-limonene, and 2-ethyl-1-hexanol. The rest of volatile compounds are presented in both, fresh and marinated loin samples.

Moreover, it can be observed there are other compounds that although they are in both type of samples, they have higher quantities in marinated loin samples than in fresh loin, they are: 3-methyl-hexane, 2-hydroxypropanoic acid ethyl ester, 1-heptanol, 2-butanol, 3-methyl-1-butanol, 2-pentyl-furano, ethyl ester hexanoic adic, decanal, 1-octanol and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol.

In addition, most of volatile compounds are in higher amount in fresh loin than in marinated loin, but only there is a significant increased ($p < 0.05$) in some of them: 2-propanone, 2-octene, 3,5,5-trimethyl-1-hexene, dimethyl disulphide, 1-(methylphenyl)-ehanone, and 1-hexanol.

3.3. Effect of different conditions of treatment and storage

In order to find out significant differences between the two types of loin, the Mann-Whitney U test was performed. The statistical parameter U was obtained for each compound and the respective z-values were calculated for being compared with the z-value in the normalized standard distribution for 95% confidence (z = 1.96). Results

Table 2
Volatile compounds identified in pork loin (see Figure 1)

Peak	Compound	I	T_{RR}	Base peak	M^+
1	2,4-dimethyl-hexane	S/L	0.081	43	114
2	3-methyl-hexane	S/L	0.088	43	100
3	2,5-dimethyl-hexane	S/L	0.093	43	114
4	2,4-dimethyl-heptane	L	0.105	43	128
5	2-propanone	S/L	0.112	43	58
6	2-octene	S/L	0.117	55	112
7	Ethyl ester acetic acid	S/L	0.139	43	88
8	2-butanone	S/L	0.146	43	72
9	2-(1-methylethoxy)-1-propanol	S/L	0.165	45	118
10	2-hydroxypropanoic acid ethyl ester	L	0.171	45	118
11	2-pentanone	S/L	0.202	43	86
12	Pentanal	S/L	0.206	44	86
13	Acetic acid ethenyl ester	S/L	0.211	43	86
14	Chloroform	S/L	0.259	83	124
15	2-butanol	S/L	0.268	45	74
16	Ethyl ester butanoic acid	S/L	0.280	71	116
17	3,5,5-trimethyl-1-hexene	S/L	0.327	57	126
18	Dimethyl disulphide	S/L	0.341	94	94
19	Hexanal	S/L	0.363	44	100
20	2-Beta-Pinene	S/L	0.383	93	136
21	2-Nitrobutane	S/L	0.428	29	103
22	3-Carene	S/L	0.501	93	136
23	Heptanal	S/L	0.673	43	114
24	dl-Limonene	S/L	0.683	68	136
25	3-methyl-1-butanol	S/L	0.802	55	88
26	2-pentyl-furane	S/L	0.852	81	140
27	Ethyl ester hexanoic acid	S/L	0.869	88	145
28	1-(methylphenyl)-ethanone	L	0.970	41	136
29	Octanal	S/L	1.086	41	128
30	1-hexanol	S/L	1.397	56	102
31	Nonanal	S/L	1.525	57	142
32	1-Heptanol	S/L	1.828	70	99
33	Decanal	S/L	1.955	43	157
34	2-ethyl-1-hexanol	S/L	1.971	57	130
35	1-octanol	S/L	2.182	41	113
36	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	S/L	2.228	71	154
37	2-decenal	S/L	2.275	43	154

I: Identification; L: Library; S: Standard; T_{RR}: relative retention time; M^+: molecular ion

of application of this test are also shown in table 3. It can be observed that the obtained z-values were higher than the critical one for 2-octene, ethyl ester acetic acid, 2-butanone, 2-butanol, 3,5,5-trimethyl-1-hexene, dimethyl disulphide, 2-Beta-Pinene, 2-Nitrobutane, 3-Carene, dl-Limonene, 3-methyl-1-butanol, 2-pentyl-furane, Ethyl ester hexanoic acid, 1-(methylphenyl)-ethanone, 1-hexanol, decanal,

Table 3
Median, minimun and maximun values (mg kg^{-1}) for the volatile compounds determined in the analyzed loin samples and Mann-Whitney U Test By variable. Type Marked tests are significant at p < 0.05000

Volatile compounds	Fresh (n = 27)			Marinated (n = 27)			U	Z
	Median	Min.	Max.	Median	Min.	Max.		
2,4-dimethyl-hexane	2916.50	31.97	20628.07	2208.48	51.01	15316.98	326.0000	0.66605
3-methyl-hexane	28.97	0.00	232.97	39.32	0.00	365.97	347.0000	–0.30275
2,5-dimethyl-hexane	3.26	0.00	43.59	2.04	0.00	9.75	322.5000	–0.72660
2,4-dimethyl-heptane	60.15	3.23	1337.75	10.02	3.56	17.99	336.0000	–0.49305
2-propanone	102.83	1.47	2107.22	31.18	1.38	405.58	312.0000	0.90825
2-octene[b]	22.96	0.00	505.59	9.22	1.91	49.80	211.0000	–2.65555
Ethyl ester acetic acid[d]	330.73	0.66	8446.78	105.46	11.27	249.71	70.0000	–5.09484
2-butanone[b]	3.52	0.00	41.02	3.82	0.00	14.97	199.0000	–2.86315
2-(1-methylethoxy)-1-propanol	873.71	0.95	13431.47	702.30	0.00	6801.07	359.0000	–0.09515
2-hydroxypropanoic acid ethyl ester	2945.82	0.00	35174.05	3330.15	0.00	25572.48	332.5000	–0.55360
2-pentanone	120.08	0.00	2929.93	7.89	0.00	174.49	356.0000	–0.14705
Pentanal	9.16	0.00	102.86	6.19	0.00	36.27	322.5000	0.72660
Acetic acid ethenyl ester	1.34	0.00	12.96	0.80	0.00	16.04	312.5000	0.89960
Chloroform	2276.19	0.00	56006.88	214.26	0.00	1399.64	297.0000	1.16775
2-butanol[c]	22.79	0.00	229.90	32.69	0.00	459.87	171.0000	–3.34755
Ethyl ester butanoic acid	24.19	0.00	255.86	13.68	0.00	86.86	356.5000	–0.13840
3,5,5-trimethyl-1-hexene[b]	11.13	0.00	255.86	5.26	0.00	65.64	202.0000	–2.81125
Dimethyl disulphide[b]	60.44	0.00	1384.74	3.13	0.00	15.10	177.0000	–3.24375
Hexanal	129.43	1.97	2798.71	22.98	4.83	110.38	274.0000	1.56565
2-Beta-Pinene[d]	0.00	0.00	0.00	5.53	0.00	17.38	81.0000	–4.90454
2-Nitrobutane[d]	0.00	0.00	0.00	11.22	0.92	31.36	0.0000	–6.30584
3-Carene[d]	0.00	0.00	0.00	12.17	0.00	26.30	40.5000	–5.60519
Heptanal	54.25	3.82	1018.52	19.55	1.51	107.44	317.0000	–0.82175
dl-Limonene[a]	0.00	0.00	0.00	3.32	0.00	25.26	229.5000	–2.33550
3-methyl-1-butanol[b]	42.69	3.16	135.75	99.05	0.00	300.43	211.0000	–2.65555
2-pentyl-furane[a]	1.66	0.00	5.84	10.29	0.00	113.51	247.0000	–2.03275
Ethyl ester hexanoic acid[b]	4.34	0.00	88.85	6.56	0.00	24.44	212.0000	–2.63825
1-(methylphenyl)-ethanone[d]	3126.88	0.00	84371.50	5.47	0.83	11.73	138.0000	–3.91844
Octanal	21.51	3.49	109.69	15.14	4.53	83.20	315.0000	0.85635
1-hexanol[c]	67.43	0.00	1770.07	4.48	0.00	13.53	154.5000	–3.63300
Nonanal	24.49	0.00	104.26	21.30	6.06	57.36	334.0000	–0.52765
1-Heptanol	1.27	0.00	11.68	3.25	0.00	51.91	362.0000	0.04325
Decanal[a]	2.64	0.00	61.31	12.04	0.00	62.47	245.5000	–2.05870
2-ethyl-1-hexanol[d]	0.00	0.00	0.00	23.17	0.00	79.53	81.0000	–4.90454
1-octanol[a]	1.08	0.00	8.81	2.49	0.00	19.08	241.0000	–2.13655
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol[d]	1.47	0.00	32.10	2.60	0.00	7.43	99.0000	–4.59314
2-decenal	4.12	0.00	59.99	0.43	0.00	2.12	283.0000	1.40995

[a] for $p < 0.05$; [b] for $p < 0.01$; [c] for $p<0.001$ and [d] for $p < 0.0001$.

2-ethyl-1-hexanol, 1-octanol and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol. The highest differences were found for ethyl ester acetic acid, 2-Beta-Pinene, 2-Nitrobutane, 3-Carene, 2-ethyl-1-hexanol and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, with z-values up to 4. The other

Table 4
Mann-Whitney U Test by variable time for both types of samples

Volatile compounds	Fresh (n = 27)			Marinated (n = 27)		
	U	Z	p-level	U	Z	p-level
2,4-dimethyl-hexane	41.00000	–2.05738	*	31.00000	2.57172	*
3-methyl-hexane	46.00000	–1.80021	–	35.50000	2.34027	*
2,5-dimethyl-hexane	64.00000	–0.87439	–	51.00000	–1.54303	–
2,4-dimethyl-heptane	76.00000	0.25717	–	56.00000	–1.28586	–
2-propanone	38.00000	2.21168	*	36.00000	–2.31455	*
2-octene	46.00000	–1.80021	–	24.00000	–2.93176	**
Ethyl ester acetic acid	78.00000	0.15430	–	34.00000	–2.41742	*
2-butanone	40.00000	–2.10881	*	3.00000	–4.01189	***
2-(1-methylethoxy)-1-propanol	68.00000	–0.66865	–	19.50000	–3.16322	**
2-hydroxypropanoic acid ethyl ester	36.00000	–2.31455	*	67.00000	0.72008	–
2-pentanone	79.00000	–0.10287	–	27.00000	–2.77746	**
Pentanal	69.00000	0.61721	–	79.00000	0.10287	–
Acetic acid ethenyl ester	70.00000	–0.56578	–	77.00000	–0.20574	–
Chloroform	67.00000	0.72008	–	12.00000	3.54898	***
2-butanol	40.00000	–2.10881	*	39.00000	–2.16025	*
Ethyl ester butanoic acid	65.00000	–0.82295	–	32.00000	2.52029	*
3,5,5-trimethyl-1-hexene	29.50000	–2.64887	**	78.50000	0.12859	–
Dimethyl disulphide	54.00000	–1.38873	–	35.00000	2.36598	*
Hexanal	57.00000	1.23443	–	63.00000	0.92582	–
2-Beta-Pinene	81.00000	0.00000	–	77.50000	–0.18002	–
2-Nitrobutane	81.00000	0.00000	–	79.00000	0.10287	–
3-Carene	81.00000	0.00000	–	64.00000	–0.87439	–
Heptanal	55.00000	1.33730	–	76.00000	0.25717	–
dl-Limonene	81.00000	0.00000	–	64.00000	0.87439	–
3-methyl-1-butanol	79.00000	0.10287	–	73.00000	–0.41148	–
2-pentyl-furane	48.00000	1.69734	–	36.50000	–2.28883	*
Ethyl ester hexanoic acid	76.00000	–0.25717	–	32.00000	2.52029	*
1-(methylphenyl)-ethanone	64.00000	–0.87439	–	65.00000	–0.82295	–
Octanal	42.00000	2.00594	*	59.00000	1.13156	–
1-hexanol	45.00000	–1.85164	–	40.00000	2.10881	*
Nonanal	39.00000	2.16025	*	73.00000	–0.41148	–
1-Heptanol	47.00000	1.74877	–	63.00000	0.92582	–
Decanal	60.00000	1.08012	–	27.00000	–2.77746	**
2-ethyl-1-hexanol	81.00000	0.00000	–	35.00000	2.36598	*
1-octanol	29.00000	2.67459	**	40.50000	–2.08310	*
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	54.00000	1.38873	–	72.00000	–0.46291	–
2-decenal	64.00000	0.87439	–	74.50000	–0.33432	–

* For $p < 0.05$; ** for $p < 0.01$ and *** for $p < 0.001$.

Table 5
Mann-Whitney U Test by variable temperature for both types of samples

Volatile compounds	Fresh (n = 27)			Marinated (n = 27)		
	U	Z	p-level	U	Z	p-level
2,4-dimethyl-hexane	59.00000	–1.13156	–	47.00000	1.74877	–
3-methyl-hexane	73.00000	0.41148	–	76.50000	0.23146	–
2,5-dimethyl-hexane	73.00000	0.41148	–	73.00000	0.41148	–
2,4-dimethyl-heptane	63.00000	0.92582	–	78.00000	0.15430	–
2-propanone	36.00000	2.31455	*	33.00000	–2.46885	*
2-octene	62.00000	–0.97725	–	57.00000	–1.23443	–
Ethyl ester acetic acid	80.00000	–0.05143	–	29.00000	–2.67459	**
2-butanone	56.00000	–1.28586	–	31.00000	–2.57172	*
2-(1-methylethoxy)-1-propanol	77.00000	0.20574	–	46.00000	–1.80021	–
2-hydroxypropanoic acid ethyl ester	69.00000	–0.61721	–	59.00000	1.13156	–
2-pentanone	80.00000	0.05143	–	38.00000	–2.21168	*
Pentanal	67.00000	0.72008	–	58.00000	–1.18299	–
Acetic acid ethenyl ester	70.00000	0.56578	–	71.00000	–0.51434	–
Chloroform	47.00000	1.74877	–	55.50000	1.31158	–
2-butanol	43.00000	–1.95451	–	39.00000	–2.16025	*
Ethyl ester butanoic acid	65.00000	0.82295	–	67.00000	0.72008	–
3,5,5-trimethyl-1-hexene	40.50000	–2.08310	*	56.00000	–1.28586	–
Dimethyl disulphide	58.00000	–1.18299	–	80.00000	0.05143	–
Hexanal	52.00000	1.49160	–	61.00000	1.02869	–
2-Beta-Pinene	81.00000	0.00000	–	59.00000	–1.13156	–
2-Nitrobutane	81.00000	0.00000	–	48.00000	–1.69734	–
3-Carene	81.00000	0.00000	–	67.00000	–0.72008	–
Heptanal	47.00000	1.74877	–	80.00000	–0.05143	–
dl-Limonene	81.00000	0.00000	–	58.00000	1.18299	–
3-methyl-1-butanol	80.00000	–0.05143	–	57.00000	–1.23443	–
2-pentyl-furane	46.50000	1.77449	–	19.00000	–3.18894	**
Ethyl ester hexanoic acid	68.00000	0.66865	–	25.50000	2.85461	**
1-(methylphenyl)-ethanone	65.00000	0.82295	–	56.00000	–1.28586	–
Octanal	36.00000	2.31455	*	67.00000	0.72008	–
1-hexanol	76.00000	–0.25717	–	73.00000	0.41148	–
Nonanal	51.00000	1.54303	–	74.00000	–0.36004	–
1-Heptanol	72.00000	0.46291	–	55.00000	1.33730	–
Decanal	63.00000	0.92582	–	3.00000	–4.01189	***
2-ethyl-1-hexanol	81.00000	0.00000	–	24.50000	2.90605	**
1-octanol	52.00000	1.49160	–	51.00000	–1.54303	–
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	52.00000	1.49160	–	79.00000	–0.10287	–
2-decenal	50.00000	1.59447	–	78.00000	0.15430	–

* For $p < 0.05$; ** for $p < 0.01$ and *** for $p < 0.001$.

volatile compounds presented z-values up to 2.5 (in absolute value), except dl-Limonene, 2-pentyl-furane, decanal and 1-octanol.

According to results obtained for the volatile compounds, the different packaging systems of non-irradiated and irradiated pork loin were treated separately in fresh and marinated loin.

As far as we are aware, studies about changes in each volatile compound with different irradiation and packaging during storage have not been

previously reported. So, this is the first time that this type of study has been done.

The end of the shelf-life of samples was established when the microbial load reach the value of 107 cfu g^{-1} (Cabeza *et al*., 2007). Accordingly, the shelf-life of both fresh and marinated loin stored under the selected conditions will be different according to the strength of the method of microbiota

Table 6
Significant differences within both types of samples (fresh and marinated) for the volatile compounds classes analyzed according to the different packaging atmosphere (air, MAP and vacuum)

Volatile compounds	Fresh (n = 27)				Marinated (n = 27)			
	H	A/V	A/MAP	V/MAP	H	A/V	A/MAP	V/MAP
2,4-dimethyl-hexane	0.18	ns	ns	ns	3.19	ns	ns	ns
3-methyl-hexane	0.70	ns	ns	ns	0.97	ns	ns	ns
2,5-dimethyl-hexane	0.03	ns	ns	ns	1.34	ns	ns	ns
2,4-dimethyl-heptane	1.10	ns	ns	ns	0.95	ns	ns	ns
2-propanone	3.56	ns	ns	ns	1.76	ns	ns	ns
2-octene	3.43	ns	ns	ns	7.16	ns	ns	ns
Ethyl ester acetic acid	0.17	ns	ns	ns	6.10	ns	ns	ns
2-butanone	1.19	ns	ns	ns	1.86	ns	ns	ns
2-(1-methylethoxy)-1-propanol	8.51	ns	ns	*	1.90	ns	ns	ns
2-hydroxypropanoic acid ethyl ester	0.68	ns	ns	ns	2.04	ns	ns	ns
2-pentanone	2.35	ns	ns	ns	1.48	ns	ns	ns
Pentanal	1.75	ns	ns	ns	1.19	ns	ns	ns
Acetic acid ethenyl ester	3.28	ns	ns	ns	0.58	ns	ns	ns
Chloroform	1.55	ns	ns	ns	1.45	ns	ns	ns
2-butanol	1.58	ns	ns	ns	6.36	ns	ns	ns
Ethyl ester butanoic acid	0.23	ns	ns	ns	3.11	ns	ns	ns
3,5,5-trimethyl-1-hexene	0.69	ns	ns	ns	9.36	ns	**	ns
Dimethyl disulphide	0.83	ns	ns	ns	0.39	ns	ns	ns
Hexanal	0.13	ns	ns	ns	1.17	ns	ns	ns
2-Beta-Pinene	0.00	ns	ns	ns	14.38	ns	*	***
2-Nitrobutane	0.00	ns	ns	ns	5.36	ns	ns	ns
3-Carene	0.00	ns	ns	ns	0.02	ns	ns	ns
Heptanal	0.11	ns	ns	ns	2.25	ns	ns	ns
dl-Limonene	0.00	ns	ns	ns	3.74	ns	ns	ns
3-methyl-1-butanol	6.92	ns	ns	ns	8.65	ns	ns	*
2-pentyl-furane	0.71	ns	ns	ns	1.66	ns	ns	ns
Ethyl ester hexanoic acid	3.01	ns	ns	ns	2.04	ns	ns	ns
1-(methylphenyl)-ethanone	7.41	ns	ns	*	4.12	ns	ns	ns
Octanal	0.07	ns	ns	ns	2.51	ns	ns	ns
1-hexanol	2.57	ns	ns	ns	0.89	ns	ns	ns
Nonanal	1.79	ns	ns	ns	1.45	ns	ns	ns
1-Heptanol	0.27	ns	ns	ns	1.63	ns	ns	ns
Decanal	0.84	ns	ns	ns	0.57	ns	ns	ns
2-ethyl-1-hexanol	0.00	ns	ns	ns	4.71	ns	ns	ns
1-octanol	0.02	ns	ns	ns	3.25	ns	ns	ns
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	2.43	ns	ns	ns	1.00	ns	ns	ns
2-decenal	0.05	ns	ns	ns	0.55	ns	ns	ns

ns, not significant;* p<0.05;** p<0.01; *** p<0.001 air: A; vacuum: V. Comparison between packaging atmospheres using Kruskal-Wallis Test.

inhibition. However, the statistical analysis showed that these differences did not affect to the integrity of most phospholipid classes. Normality of the variables in the comparison groups was studied by means of Kolmogorov - Smirnov - Lilliefors test. In light of the results of this test, non parametric test, such as Kruskal-Wallis and Mann-Whitney U test were used for all between-group comparisons.

Table 7
Significant differences within both types of samples for the volatile compounds analyzed according to the different irradiation doses (0, 1 and 2 kGy)

Volatile compounds	Fresh(n=27)				Marinated (n=27)			
	H	0/1	0/2	1/2	H	0/1	0/2	1/2
2,4-dimethyl-hexane	0.60	ns	ns	ns	2.03	ns	ns	ns
3-methyl-hexane	1.93	ns	ns	ns	1.59	ns	ns	ns
2,5-dimethyl-hexane	0.64	ns	ns	ns	2.05	ns	ns	ns
2,4-dimethyl-heptane	3.15	ns	ns	ns	0.96	ns	ns	ns
2-propanone	1.11	ns	ns	ns	0.00	ns	ns	ns
2-octene	0.14	ns	ns	ns	0.03	ns	ns	ns
Ethyl ester acetic acid	5.81	ns	ns	ns	0.26	ns	ns	ns
2-butanone	0.02	ns	ns	ns	0.35	ns	ns	ns
2-(1-methylethoxy)-1-propanol	3.83	ns	ns	ns	0.74	ns	ns	ns
2-hydroxypropanoic acid ethyl ester	0.30	ns	ns	ns	0.11	ns	ns	ns
2-pentanone	0.11	ns	ns	ns	1.54	ns	ns	ns
Pentanal	0.52	ns	ns	ns	4.90	ns	ns	ns
Acetic acid ethenyl ester	0.69	ns	ns	ns	0.34	ns	ns	ns
Chloroform	1.88	ns	ns	ns	0.65	ns	ns	ns
2-butanol	1.62	ns	ns	ns	3.18	ns	ns	ns
Ethyl ester butanoic acid	0.54	ns	ns	ns	0.99	ns	ns	ns
3,5,5-trimethyl-1-hexene	2.59	ns	ns	ns	0.93	ns	ns	ns
Dimethyl disulphide	1.50	ns	ns	ns	10.00	ns	*	ns
Hexanal	1.88	ns	ns	ns	0.26	ns	ns	ns
2-Beta-Pinene	0.00	ns	ns	ns	0.40	ns	ns	ns
2-Nitrobutane	0.00	ns	ns	ns	1.93	ns	ns	ns
3-Carene	0.00	ns	ns	ns	2.74	ns	ns	ns
Heptanal	1.91	ns	ns	ns	0.43	ns	ns	ns
dl-Limonene	0.00	ns	ns	ns	0.09	ns	ns	ns
3-methyl-1-butanol	3.24	ns	ns	ns	0.64	ns	ns	ns
2-pentyl-furane	0.67	ns	ns	ns	0.33	ns	ns	ns
Ethyl ester hexanoic acid	0.20	ns	ns	ns	0.24	ns	ns	ns
1-(methylphenyl)-ethanone	0.79	ns	ns	ns	0.26	ns	ns	ns
Octanal	0.85	ns	ns	ns	0.44	ns	ns	ns
1-hexanol	4.60	ns	ns	ns	0.06	ns	ns	ns
Nonanal	4.17	ns	ns	ns	0.68	ns	ns	ns
1-Heptanol	1.45	ns	ns	ns	2.65	ns	ns	ns
Decanal	2.28	ns	ns	ns	0.82	ns	ns	ns
2-ethyl-1-hexanol	0.00	ns	ns	ns	0.39	ns	ns	ns
1-octanol	1.72	ns	ns	ns	0.27	ns	ns	ns
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	3.34	ns	ns	ns	0.10	ns	ns	ns
2-decenal	4.26	ns	ns	ns	1.72	ns	ns	ns

ns, not significant;* $p<0.01$. Comparison between irradiation doses using Kruskal-Wallis Test.

In order to find out significant differences between different volatile compounds for two times values used and both types of samples prepared a Mann-Whitney U test was performed. The statistical parameter U was obtained for each compound and the respective z-values were calculated for being compared with the z-value in the normalized standard distribution for 95% confidence. Table 4 shows the results of this application. It can be observed that significant differences ($p < 0.01$) have been found for 3,5,5-trimethyl-1-hexene and 1-octanol in fresh loin, 2,4-dimethyl-hexane, 2-propanone, 2-butanone, 2-hydroxypropanoic acid ethyl ester, 2-butanol, octanal and nonanal also presents significant differences ($p < 0.05$), all showing a higher level in shelf-life, except 1-octanol, octanal and nonanal. In marinated loin several compounds show significant differences, 3,5,5-trimethyl-1-hexene, 3-methyl-hexane, 2-propanone, ethyl ester acetic acid, 2-butanol, ethyl ester butanoic acid, dimethyl disulfide, 2-pentyl-furane, ethyl ester hexanoic acid, 2-ethyl-hexanol and octanol ($p < 0.05$); 2-pentanone and decanal ($p < 0.01$); 2-octene ($p < 0.005$); chloroform ($p < 0.0005$) and 2-butanone ($p < 0.0001$), showing a lower level in shelf-life, except 2-propanone, 2-octene, ethyl ester acetc acid, 2-butanone, 2-pentanone, decanal and 1-octanol. These increases in aldehydes and ketones can due to oxidation of fatty acids.

In Table 5, the results obtained from Mann-Whitney U test to study the effect of temperature are presented for both kinds of samples (fresh and marinated). Two temperatures (4 and 8 °C) have been applied for the storage. Only significant differences ($p < 0.05$) are observed for 2-propanone, 3,5,5-trimethyl-1-hexene and octanal in fresh loin. There are not references about the effect of temperature in volatile compounds. In marinated loin, significant differences have been found in a large number of compound, 2-propanone, 2-butanone, 2-butanol ($p < 0.05$), ethyl ester acetic acid, 2-penthyl-furane and ethyl ester hexanoic acid ($p < 0.01$), possibly because of the additives used.

As well, the effect of packaging atmosphere (air, MAP and vacuum) has been study applying a Kruskal-Wallis test. The results are in Table 6 and it can be deduced that there are significant differences ($p < 0.05$) between MAP and vacuum for 2-(1-methylethoxy)-1-propanol in the case of fresh loin, being the mean value highest in vacuum. This fact has not explanation and no references about it have been found. On the other hand, the packaging atmosphere has a major effect in volatile compounds from marinated loin, for 2-beta-pinene there are significant differences ($p < 0.05$) between MAP and air and ($p < 0.001$) between MAP and vacuum. Also, there are significant differences ($p < 0.01$) between MAP and air for 3,5,5-trimethyl-1-hexene.

Finally, several irradiation doses have been used for both types of loin (0, 1 and 2 kGy). The effect of this was also studied using a Kruskal-Wallis test, whose data are presented in Table 7. No effect of the irradiation doses (until 2 kGy) on changes in the individual volatile compounds in fresh loin was observed which is valuable result since E-beam may be applied as an useful tool to extend the shelf-life of fresh loin without alterations. Only significant differences ($p < 0.05$) between 0 and 2 kGy are observed for dimethyl disulfide in marinated loin.

CONCLUSIONS

A study of the effect of E-beam irradiation and packaging on the volatile compounds from fresh and marinated pork loin has been carried out. Some differences were found between samples, namely in terpenes which only were detected in marinated sample due to the seasoning, which included paprika, source of those volatiles. Minor differences were found between the three type of packaging (air, vacuum and carbon dioxide) and storage temperatures (2 and 8 °C). However, in the context of the objective of the present work, the result of most concern is that no effect of the irradiation doses was found on changes in the individual volatile compounds in both products, even when 2 kGy was applied. Thus, the E-beams may be a very useful tool to extend the shelf-life of fresh and marinated pork loin. Additionally, this technology reduces the number of pathogens to negligible levels.

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