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Evaluation of 2-Alkylcyclobutanones in Irradiated Cured Pork Products during Vacuum-Packed Storage

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The 2-Alkylcyclobutanones (2-ACBs) content was determined in three Italian cured pork products (salame Milano, coppa, and pancetta) irradiated at different targeted irradiation doses (2, 5, and 8 kGy) during vacuum-packed storage. Among 2-ACBs, three different compounds were investigated, namely, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and 2-(tetradec-5′-enyl)cyclobutanone. 2-ACBs were absent from the nonirradiated samples, whereas their content increased with irradiation dose. Their presence was recorded occasionally at 2 kGy and constantly at higher irradiation doses (5 and 8 kGy). The plot of 2-ACBs content against targeted irradiation doses showed an exponential relationship. The effect of vacuum-packed storage time on the 2-ACBs content was dependent on the irradiation dose. During vacuum-packed storage for up to 60 days, the 2-ACBs content remained unchanged in the cured pork products irradiated at 2 and 5 kGy, whereas a significant increase was observed in the pork products irradiated at 8 kGy.

KEYWORDS: 2-Alkylcyclobutanones; irradiation; cured pork products; salame Milano; coppa; pancetta; vacuum-packed storage

INTRODUCTION

In 2005, Italian cured pork products accounted for 1147800 tons, and the export volume achieved around 8.3% of the global production, equivalent to 705 million Euros (*1*). In recent years, Italian cured pig meat producers have expressed a growing interest in the feasibility of irradiation technology to improve the safety of meat products, in particular of those intended for export in some markets where public health and regulatory agencies have established a zero tolerance in ready-to-eat foods for some pathogens like *Listeria monocytogenes* (*2*).

In the European Union, the Community positive list of foods and food ingredients that may be treated with ionizing radiation, established by the Directives 1999/2/EC (*3*) and 1999/3/EC (*4*), includes up to now the single category of "dried aromatic herbs, spices and vegetable seasoning", although existing authorizations in certain Member States allow the irradiation of a number of foodstuffs. However, treatment with ionizing radiation of meat and meat products is not authorized in the European Union, except for chicken meat (The Netherlands), poultry (France and United Kingdom), and mechanically recovered chicken meat (France).

Irradiation is well-known to be able to increase food safety by reduction of spoilage and pathogenic microorganisms, and its use is gradually increasing worldwide. Treatment of some foodstuffs with ionizing radiations is advantageous for pathogen control in HACCP-based systems or in combination with other methods of food preservation, such as modified atmosphere or vacuum packaging (*5*).

On the other hand, irradiation treatment brings about chemical changes that could affect the nutritional and sensorial adequacy of food. The changes induced in lipids, proteins, and vitamins have been widely discussed (*6*). One of the major concerns with irradiation treatment is the formation of radiolytic compounds, in particular 2-alkylcyclobutanones (2-ACBs).

2-ACBs are cyclic ketones with a ring at four carbon atoms and an alkyl chain in position two. They are formed by the loss of an electron from the oxygen on the carbonyl of a free fatty acid or triglyceride, followed by a rearrangement process to produce 2-ACBs specific to the parent fatty acid. The resulting compounds have the same number of carbon atoms as the precursor fatty acids.

Le Tellier and Nawar (*7*) first identified 2-ACBs as radiolytic products from pure triglycerides irradiated at high doses (60 kGy). Further researches have produced evidence that 2-ACBs are formed by radiolysis in fat-containing foods exposed to irradiation treatment by electron beams, X-rays, or γ-radiation of ^{60}Co or ^{137}Cs, and they can be specifically detected in

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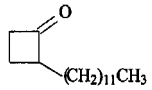
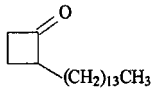
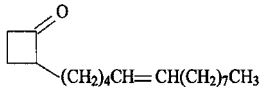


2-dodecylcyclobutanone (2-dDCB)



2-tetradecylcyclobutanone (2-tDCB)



2-(tetradec-5'-enyl)cyclobutanone (2-tDeCB)

Figure 1. Chemical structure of 2-ACBs investigated.

irradiated foods as markers of this processing technique. 2-ACBs have never been detected in nonirradiated food stored or treated by other food processes such freezing, pasteurization, microwave treatment, oven heating, UV irradiation, high-pressure treatment, packaging under vacuum, or carbon dioxide (*8*−*10*).

2-ACBs have been detected in different types of irradiated fat-containing foods: Brie and Camembert cheese (*11*, *12*); liquid whole egg (*9*, *13*); fish, crustacea, and mollusca (*14*−*16*); and chicken meat and beef (*8*, *10*, *17*−*20*). Referring to pig meat, raw pork loin (*21*−*24*), pork patties (*25*, *26*), cooked pork sausage (*27*−*29*), and Chinese sausage (*30*) have been widely investigated for physicochemical, microbiological, and sensory changes induced by irradiation treatment. However, some studies have been reported on the determination of 2-ACBs in raw pork (*31*−*35*), but no literature data are available on irradiated cured pork products. As well as unique radiolytic products and markers for the detection of irradiated food, concerns about the safety of 2-ACBs are of great interest for a risk assessment for human health associated with the consumption of these molecules present in irradiated fat-containing foods (*36*). Even though there are ample data showing no adverse effects of irradiated foods, limited and contradictory data are available on the toxicological and metabolic properties of pure 2-ACBs. The fate of 2-ACBs after consumption has been recently studied in rats by their quantification in adipose tissue and feces (*13*) and by investigating their urinary metabolites (*37*).

Therefore, this study was performed to determine 2-ACBs induced by treatment with ionizing radiation at different irradiation doses of three Italian cured pork products (salame Milano, coppa, and pancetta) during vacuum-packed storage. These findings are compulsory to make possible the exposure assessment, within the risk analysis, related to irradiated fat-containing food.

MATERIALS AND METHODS

Chemicals. All reagents and solvents were of analytical grade. A fatty acid methyl esters standard mixture was supplied by Sigma-Aldrich (Milan, Italy). Boron trifluoride−methanol complex (14% w/w BF_3) was obtained from VWR International (Milan, Italy).

2-Dodecylcyclobutanone (2-dDCB), 2-tetradecylcyclobutanone (2-tDCB), and 2-(tetradec-5′-enyl)cyclobutanone (2-tDeCB) (**Figure 1**) were purchased from Fluka (Milan, Italy). 2-Cyclohexylcyclohexanone (2-cHCH), used as an internal standard, was purchased from Alfa Aesar (Karlsruhe, Germany). Florisil (60/100 mesh) was supplied by Supelco (Sigma-Aldrich). Florisil was heated overnight at 550 °C and kept well-sealed after cooling; before use, it was deactivated by heating for 5 h at 130 °C in a dry oven and cooling in a desiccator. Afterward, 20% distilled water (w/w) was added and the mixture was shaken for 20 min. The mixture was stored at room temperature for 10−12 h. Florisil deactivated in this way was used for 3 days.

Sample Preparation. Three Italian cured pork products, salame Milano, coppa, and pancetta, were investigated. For each type of cured pork product, 35 whole pieces were supplied by a local manufacturer.

Salame Milano is a typical Italian minced, dry cured, and fermented sausage. The processing technology of the products investigated in this study was the same as described by Novelli et al. (*38*). The average weight (±standard error) of salame Milano supplied ($n = 10$) was 3.46 ± 0.125 kg. Coppa owes its name to the part of the pig from which it is obtained, that is, the back of the neck. It is typical of Parma and Piacenza provinces, although it is also produced in other parts of Italy. The shape of matured coppa, stuffed in bovine bladder, is cylindrical and pointed at the ends, with a firm and compact texture. The processing technology of coppa supplied for the present investigation was the same as described by Zanardi et al. (*39*). The average weight (±standard error) of coppa supplied ($n = 10$) was 1.90 ± 0.139 kg. Pancetta is produced in different parts of Italy. It is manufactured from the pork belly, located between the retrosternal and the inguinal regions, properly trimmed. The most traditional version is rolled, but many other varieties exist, including those in which the pork belly is flattened or smoked. The processing technology of pancetta supplied for the present investigation was the same as described by Bellatti et al. (*40*). The average weight (±standard error) of pancetta supplied ($n = 10$) was 2.58 ± 0.120 kg.

The cured pork products, belonging to the same batch of standard production, were singularly vacuum-packed into oxygen-impermeable nylon/polyeyhylene bags (Polinyl 145 μm, O_2 trans. rate 8−10 $cm^3/m^2/24$ h/atm) at the end of maturation time.

Irradiation. For each type of product, five whole pieces were randomly chosen for comparison purposes (nonirradiated control samples, $n = 5$) and ten whole pieces were randomly allotted in three groups intended for irradiation treatment at 2, 5, and 8 kGy irradiation doses, respectively. The whole pieces of each group were arranged in a corrugated cardboard box in a single layer prior to irradiation. Irradiation was performed using a Linear Electron Accelerator (Circe III, Thomson-CSF Linac, St. Aubin, France), with an energy level of 10 MeV and a power level of 25 kW. The samples were irradiated at targeted irradiation doses of 2, 5, and 8 kGy by operating at an average dose rate of 90.0 kGy/min. Two alanine dosimeters were positioned, respectively, to the top and bottom surfaces of each corrugated cardboard box. The absorbed dose was within ±5% of the targeted dose.

The vacuum-packed samples, arranged in cardboard box, were kept at 5 ± 1 °C up to the irradiation treatment and exposed to ambient temperature (20 ± 1 °C) during treatment. The total time samples without refrigeration did not exceed 10 min. Immediately after the treatment, irradiated samples were stored at 5 ± 1 °C. They were analyzed within 48 h of being irradiated (0 day, $n = 5$) or stored for 60 days at 5 ± 1 °C prior to analysis (60 days, $n = 5$).

Fatty Acid Profile. The fatty acid profile was determined only in nonirradiated control samples ($n = 5$). The fatty acid composition was carried out on total lipids extracted according to Folch et al. (*41*) after methyl-esterification in the presence of boron trifluoride as a catalyst (*42*). The recovered fatty acid methyl esters (FAMEs) were analyzed using a gas chromatograph HP 6890 (Agilent Technologies, Milan, Italy) equipped with a capillary fused silica column HP INNOWax (Agilent Technologies) 30 m, 0.25 mm i.d. with a 0.25 μm polyethylene glycol stationary phase, a split/splitless injector, and a flame ionization detector. The latter two were kept at constant temperatures of 260 and 270 °C, respectively. The column oven temperature was programmed from 50 (2 min hold) to 220 °C at 4 °C min^{-1}; the final temperature of 200 °C was held for 22 min. The carrier (30 cm s^{-1} average velocity) was nitrogen (99.9995% purity, Sapio S.r.l., Monza, Italy) set at 13 psi constant pressure. The injection volume was 1 μL, and the injection was performed in split mode. ChemStation software (Agilent Technologies) was used as an acquisition and data-processing system. Peaks were identified by comparison with known FAME standard mixture (Sigma-Aldrich). Quantitation of methyl esters was accomplished using methylundecanoate (C11:0) as an internal standard. Fatty acids were expressed in gravimetric concentrations (mg g^{-1} fat).

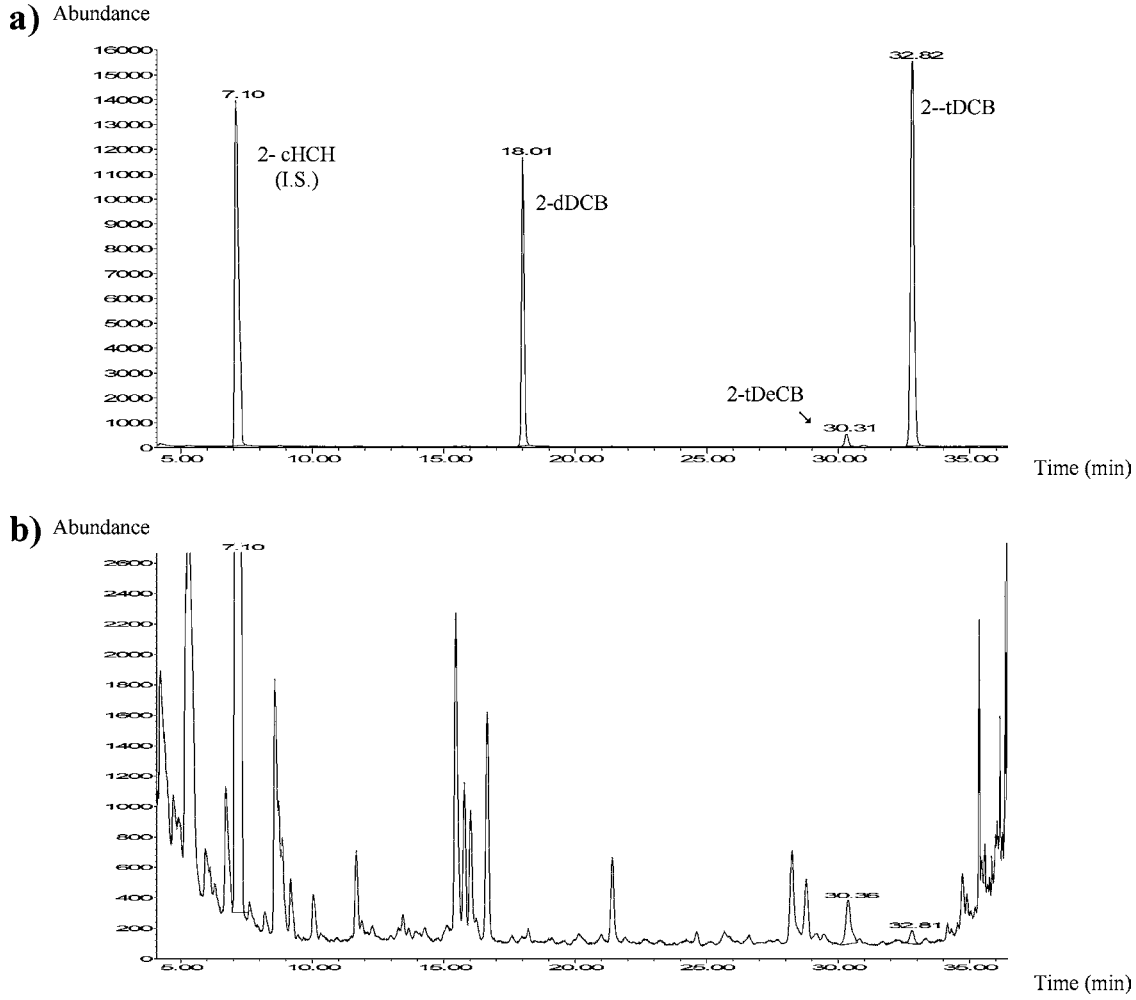


Figure 2. Chromatographic profiles obtained for the detection of 2-ACBs (selected ion monitoring of the ion *m*/*z* 98). (**a**) Reference standard mixture and (**b**) pancetta irradiated at 2 kGy.

2-ACBs. The determination of 2-ACBs was performed following the procedure described by the European Standard EN 1785 (*43*). Briefly, this official method involves Soxhlet extraction of fat from a homogenized sample with hexane, fractionation by Florisil column chromatography with 1% diethyl ether in *n*-hexane, and gas chromatography/mass spectrometry analysis in the selected ion monitoring (SIM) mode.

The fraction containing 2-ACB was analyzed by a chromatograph GC 6890N (Agilent Technologies) coupled with a single quadrupole mass selective detector MSD 5973 Network (Agilent Technologies). The gas chromatograph was fitted with a DB5-MS capillary column (Agilent Technologies) 30 m, 0.25 mm i.d. with a 0.25 μm stationary phase (5% diphenyl- and 95% dimethylpolysiloxane). The GC was equipped with a split/splitless inlet held at 250 °C. The column temperature program was as follows: initial temperature, 120 °C, held for 1 min; ramp at 15 °C min^{-1} to 160 °C, held for 2.7 min; ramp at 0.5 °C min^{-1} to 175 °C, held for 30 min; ramp at 27 °C min^{-1} to a final temperature of 250 °C, held for 3 min. The injection volume was 1 μL, and the injection was performed in splitless mode. The carrier (1 mL min^{-1} flow) was helium (99.9995% purity, Sapio S.r.l.). The transfer line and ion source were held at 280 °C throughout the run. The mass spectrometer was operated in electron impact ionization mode and positive ions (emission current, 30 μA; electron multiplier voltage, 1200 V). The mass spectra were recorded in SIM mode. The ions *m*/*z* 98 and 112 were monitored for 2-dDCB and 2-tDCB; the ions *m*/*z* 67, 81, 98, and 109 were monitored for 2-tDeCB; and the ions *m*/*z* 83 and 98 were monitored for 2-cHCH. The compounds were identified by comparing retention times and ion ratios with those of the corresponding standards analyzed at the same time. Quantitative determination of 2-ACBs was accomplished using 2-cHCH as an internal standard and by monitoring the ion *m*/*z* 98. The concentration of 2-ACBs was expressed in micrograms per gram of pork product (μg g^{-1}).

ChemStation software (Agilent Technologies, Milan, Italy) was used as the acquisition and data processing system. **Figure 2** shows the chromatographic profiles of a reference standard mixture and of a sample of pancetta.

Statistical Analysis. Summary statistics (means and standard errors of the mean) were computed for each dependent variable. 2-ACB contents were subjected to two-way analysis of variance to test the significance of the effect of the targeted irradiation dose and vacuum-packed storage time. For statistical purposes, 2-ACBs values under the limit of quantification (LOQ) were treated as 0 μg g^{-1}. Mean values were separated at, or below, the 5% probability level using the Scheffé post hoc test. All statistical computations were performed using the Statistica software package (Release 5, 1997; StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

The average content (±standard error) of total lipids of salame Milano, coppa, and pancetta were 31.2 ± 1.46, 20.0 ± 5.08, and 33.9 ± 3.94%, respectively. These values fall in a normal range for commercial products of such a type. According to Gadgil et al. (*20*), the amount of fat may not be a factor affecting 2-ACBs formation, in particular, those of 2-dDCB. In the range of 15−25% fat content, they did not observe significant differences in the amount of 2-dDCB in irradiated beef patties, thereby supposing there might be an upper threshold beyond which the amount of fat does not affect 2-ACBs formation.

The most represented fatty acids in the lipid extracted from the three cured pork meat products, in descending order of

Table 1. Fatty Acid Composition (mg g^{-1} Fat) for Salame Milano, Coppa, and Pancetta

fatty acid[a]	salame Milano	coppa	pancetta
8:0	0.07 ± 0.01	0.04 ± 0.01	0.08 ± 0.03
10:0	0.69 ± 0.04	0.60 ± 0.10	0.71 ± 0.03
12:0	0.96 ± 0.07	1.20 ± 0.99	1.27 ± 0.75
14:0	13.2 ± 0.71	13.9 ± 3.95	13.8 ± 1.78
14:1 (*n*-5)	0.16 ± 0.01	0.14 ± 0.06	0.14 ± 0.01
16:0	224 ± 13.4	237 ± 23.0	226 ± 4.88
16:1 (*n*-7)	20.6 ± 0.94	16.6 ± 4.58	19.4 ± 2.12
18:0	123 ± 10.8	156 ± 41.1	127 ± 25.6
18:1 (*n*-9)	354 ± 17.5	331 ± 21.9	339 ± 17.3
18:2 (*n*-6)	108 ± 6.02	132 ± 12.5	115 ± 42.3
18:3 (*n*-3)	5.36 ± 0.26	7.57 ± 1.68	5.25 ± 2.61
20:0	1.72 ± 0.16	1.87 ± 0.44	1.81 ± 0.23
20:1 (*n*-6)	8.13 ± 0.62	8.35 ± 1.93	7.45 ± 0.54
20:2 (*n*-6)	4.94 ± 0.35	5.87 ± 1.24	4.94 ± 1.79
20:4 (*n*-6)	2.96 ± 0.17	3.93 ± 0.56	2.18 ± 0.98
20:5 (*n*-3)	0.21 ± 0.02	0.24 ± 0.05	0.34 ± 0.42
22:5 (*n*-3)	0.94 ± 0.10	1.25 ± 0.15	0.47 ± 0.28

[a] Values represent means ± standard errors ($n = 5$).

gravimetric concentration, were oleic, palmitic, and stearic acids [18:1 (*n*-9), 16:0, and 18:0, respectively] (**Table 1**). The order of importance and the level of the main fatty acids observed in this study were similar to those reported by others in the same types of cured meat products (*39*, *44*).

When exposed to irradiation, palmitic, stearic, and oleic acids are converted to their corresponding 2-ACBs, namely, 2-dDCB, 2-tDCB, and 2-tDeCB. The three compounds were all chosen for this investigation (**Figure 1**).

Referring to 2-tDeCB, considerable analytical difficulties were encountered by several laboratories in the detection of this molecule in irradiated food samples; this is certainly the reason why until now this compound has not been included in the European Standard EN 1785 (*43*). Only a few studies were designed for the analysis of 2-tDeCB in irradiated meat (*19*, *45*), and no interlaboratory studies were organized to validate its use for food irradiation detection. According to Horvatovich et al. (*45*), the detection of 2-tDeCB should only be preferred over 2-dDCB when the concentration of its precursor oleic acid is at least three times higher than that of palmitic acid. Despite the fact that the ratio of the concentrations between oleic and palmitic acid in the investigated cured pork products was much lower that that indicated, 2-tDeCB was included in the present study because the knowledge of the amount of all of the 2-ACBs induced by the irradiation treatment and their changes during vacuum-packed storage is mandatory to make possible an exposure assessment, within the risk analysis, related to irradiated fat-containing food.

From the analytical point of view and in line with other authors (*45*, *46*), 2-tDeCB presented a more intense fragmentation pattern when compared to 2-dDCB and to 2-tDCB, resulting in an important reduction of the ion *m*/*z* 98. On the basis of the signal-to-noise ratio, the limit of detection (LOD) of 2-tDeCB was consequently 10 times higher than that for both 2-dDCB and 2-tDCB (0.2 vs 0.02 $\mu g\ g^{-1}$ sample, respectively), and the LOQ was ≈13 times higher (0.4 vs 0.03 $\mu g\ g^{-1}$ sample, respectively).

At doses included between 2 and 10 kGy, the irradiation treatment of foodstuffs is used, essentially to reduce bacterial contamination and to eliminate pathogenic microorganisms. In 1981, 10 kGy was set as the maximum dose considered to be safe and wholesome by the Join Expert FAO/IAEA/WHO Committee on the wholesomeness of irradiated food (*47*). However, in 1997, a Joint FAO/IAEA/WHO Study Group concluded that food irradiated to any dose appropriate to achieve the intended technological objective was both safe to consume and nutritionally adequate and, furthermore, that no upper dose limit needed to be imposed (*48*). More recently, the suggested removal of the upper limit of 10 kGy was not accepted by the Scientific Committee on Food of European Commission due to the very limited toxicological studies carried out with foods irradiated at doses above 10 kGy; the same Committee was of the opinion that it is appropriate to specify a maximum dose for the treatment of certain food products by ionizing radiation and that irradiated foodstuffs should continue to be evaluated individually, taking into account the technological need and their safety (*49*). The targeted irradiation doses 2, 5, and 8 kGy of this study were chosen mainly for microbiological purposes. However, the results of the effect of irradiation doses on technological, spoilage, and pathogenic flora of cured meat products are not shown and the microbiological aspects are not discussed in this paper because this latter is focused on the study of 2-ACBs induced by irradiation treatment.

2-ACBs were under the LOD in the nonirradiated cured pork products investigated. At 2 kGy, the three compounds were quantified only in a few samples because, in some pork products, they were either under the LOQ or even the LOD (**Table 2**). At 5 and 8 kGy, 2-dDCB, 2-tDCB, and 2-tDeCB were found in all of the pork products, except for 2-tDeCB, which was not constantly detected in salame Milano and coppa irradiated at 5 kGy. Pancetta showed the highest content of the three molecules at all three irradiation doses. No comparison was possible with literature data due to the lack of information on 2-ACBs in irradiated cured pork products. However, the values reported in **Table 2**, referred to as pork products after the irradiation treatment (0 day), are largely in line with those either found in the literature or expressly calculated by literature data for irradiated raw pork (*33*, *35*) and broadly in accordance with the findings for 2-dDCB in ground beef patties in the works of Gadgil et al. (*19*, *20*).

Taking into account the higher LOD and LOQ of 2-tDeCB as compared to those of 2-dDCB and 2-tDCB, the amount of 2-tDeCB formed at the lower doses might possibly have been too small to be detected. As already mentioned, the presence of this molecule became constant from 5 kGy targeted irradiation dose only in pancetta and from 8 kGy in all of the pork products investigated. Among 2-ACBs, 2-tDeCB showed the highest concentration when detected. Similar observations were made by Park et al. (*33*) in relation to raw pork, by Kim et al. (*16*) in dried shrimps both irradiated from 0.5 up to 10 kGy, and by Horvatovich et al. (*45*) in 10 kGy irradiated poultry meat.

Previous studies on irradiated raw meat (*8*, *19*, *20*, *33*, *45*, *50*) have reported that the concentration of 2-ACBs increased linearly with the irradiation dose up to 10 kGy. However, an exponential increase was observed in the present study. The concentration of 2-dDCB and 2-tDCB measured immediately after the irradiation treatment (0 day) as a function of the irradiation doses evolved according to an exponential relationship in all of the investigated meat products (**Figure 3**). The correlation coefficient r^2 of the exponential relationship between 2-dDCB content and irradiation dose was 0.72 in salame Milano, 0.9347 in pancetta, and 0.5181 in coppa; the correlation coefficient r^2 of the exponential relationship between 2-tDCB content and irradiation dose was 0.7845 in salame Milano, 0.8783 in pancetta, and 0.4109 in coppa. The pathway of increase of 2-tDeCB was not considered because this molecule was above the LOD only in the pork products irradiated at 8 kGy and in some samples irradiated at 5 kGy.

Table 2. 2-ACBs Content (μg g^{-1}) of Irradiated Salame Milano, Coppa, and Pancetta after the Irradiation Treatment (0 Day) and after 60 Days of Vacuum-Packed Storage (60 Days)

			irradiation dose (ID)						
pork product	2-ACBs[a,b]	storage time (ST) (days)	2 kGy	5 kGy	8 kGy	RMSE[c]	ID	ST	ID × ST
salame	2-dDCB	0	traces[d]	0.026	y 0.076	0.060	***	***	***
		60	traces b	0.068 b	x 0.346 a				
	2-tDCB	0	traces	0.048	y 0.080	0.058	***	***	***
		60	traces b	0.088 b	x 0.330 a				
	2-tDeCB	0	ND[e] b	traces b	y 0.592 a	0.387	***	***	***
		60	ND b	traces b	x 2.346 a				
coppa	2-dDCB	0	0.044 b	0.052 b	y 0.130 a	0.034	***	*	***
		60	ND b	0.074 b	x 0.256 a				
	2-tDCB	0	0.036	0.062	y 0.114	0.047	***	**	***
		60	ND b	0.080 b	x 0.306 a				
	2-tDeCB	0	ND	n.d.	y 0.442	0.258	***	***	***
		60	ND b	traces b	x 1.724 a				
pancetta	2-dDCB	0	0.052 b	0.124 ab	y 0.262 a	0.087	***	*	***
		60	traces b	0.114 b	x 0.568 a				
	2-tDCB	0	traces b	0.102 b	y 0.238 a	0.068	***	*	**
		60	traces b	0.092 b	x 0.418 a				
	2-tDeCB	0	ND b	0.868 b	2.516 a	0.680	***	NS	*
		60	traces b	0.430 b	3.814 a				

[a] Values represent means of five samples for each pork product. [b] ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; NS, not significant; mean values in the same row followed by different letters differ significantly; mean values within a column and 2-ACBs preceded by different letters differ significantly (Scheffé, $P \leq 0.05$). [c] Root mean squared error. [d] Detected in less than three samples out of five. [e] Under the LOQ and/or LOD.

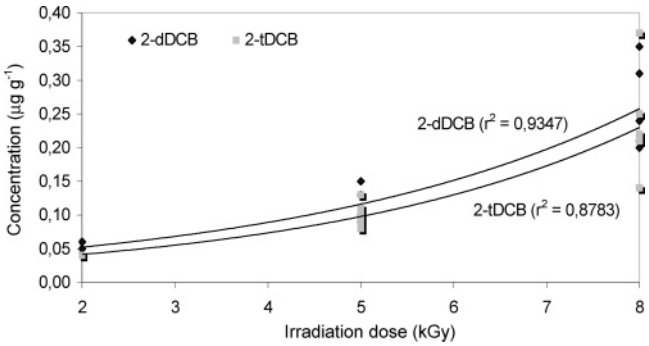


Figure 3. Effect of targeted irradiation dose (2–8 kGy) on the concentration (μg g^{-1}) of 2-dDCB and 2-tDCB in pancetta after the irradiation treatment (0 day).

The inconsistency between the relationship observed in this study and those reported by other authors could be ascribed to ingredients and additives that are present in cured meat products, but absent in raw meat, and could have affected the formation of radiolytic compounds. The cured pork products investigated contain some additives with pro-oxidant (sodium chloride) and antioxidant (sodium nitrate, sodium nitrite, and ascorbic acid) properties. Because the radiolysis proceeds by a radical mechanism, it cannot be excluded that those additives can affect the formation of 2-ACBs in cured pork products depending on the irradiation dose. However, this hypothesis should be substantiated by further investigations.

The formation of radiolytic compounds during irradiation can be sensitive to the atmosphere in which the process is carried out. This has been widely demonstrated with the lipid oxidation products, whereas very few studies have considered whether and how the 2-ACBs formation is affected by environmental conditions. The results provided by Stevenson et al. (*50*) failed to demonstrate an effect of the packaging atmosphere (air, vacuum, and CO_2) on the formation of 2-dDCB in chicken meat irradiated at 2.5 kGy. Similar observations were made by Ndiaye et al. (*10*) who showed that the irradiation up to 3.1 kGy of chicken meat under reduced oxygen pressure did not induce an appreciable lowering of the concentration of the 2-ACBs formed. However, given the feasibility of the irradiation treatment on convenience pork products, the vacuum package was chosen for this study.

The effect of vacuum-packed storage time on the 2-ACBs content gave origin to inconsistent results probably due to the statistically significant interaction between the effect of storage time and the irradiation dose (**Table 2**). In salame Milano, coppa, and pancetta irradiated at both 2 and 5 kGy, the storage up to 60 days did not induce a statistically significant increase of the level of the three 2-ACBs investigated; at these irradiation doses, the content of 2-dDCB, 2-tDCB, and 2-tDeCB remained unchanged during the vacuum storage in all of the pork products. However, the increase of the three compounds was statistically significant in the pork products irradiated at 8 kGy, with the exception of pancetta in which unvaried concentrations of the 2-tDeCB were recorded.

No comparison was possible with literature due to the lack of information and scientific evidence on the effect of vacuum-packed storage on radiation-induced 2-ACBs formation in irradiated meat. However, the unchanged concentration of the 2-ACBs in the pork products irradiated at 2 and 5 kGy during vacuum-packed storage and the trend of increase observed in those irradiated at 8 kGy did not agree with the general picture reported in the literature for the storage of irradiated meat in the presence of air. Crone et al. (*8*) observed a reduction in the amount of 2-dDCB in chicken meat wrapped in commercial plastic food wrap after a storage of 18 days at 4 °C, as well as Ndiaye et al. (*10*), who reported losses of 52% for 2-dDCB and of 48% for 2-tDeCB in chicken meat irradiated up to 3 kGy, after 1 month of storage in the presence of air. More recently, Horvatovich et al. (*45*) observed losses of 2-ACBs between 31 and 65% in 10 kGy irradiated lyophilized poultry meat placed in plastic bags and stored at 4 and 25 °C up to 28 days. On the basis of the data mentioned, some authors argued that the losses of 2-ACBs observed during the storage probably

result from a sheer phenomenon of volatilization, which explains why the losses of 2-ACBs increase as the hydrocarbon chain of these compounds is shortened (*10*). According to Hamilton et al. (*51*), the losses might also result from an oxidation of the 2-ACBs to lactones. In the authors' opinion, the high degree of impermeability of the plastic film used for the vacuum-packed storage of this study could be responsible of the barrier effect against the phenomenon of volatilization of 2-ACBs.

In conclusion, in the Italian cured pork products investigated (salame Milano, coppa, and pancetta), the content of 2-ACBs increased with irradiation dose. Their presence was recorded occasionally at 2 kGy and constantly at higher radiation doses (5 and 8 kGy). Differently from previous studies related to irradiated meat, the plot of 2-ACBs content against targeted irradiation doses showed an exponential relationship. The effect of vacuum-packed storage time on the 2-ACBs content was dependent on the irradiation dose. During vacuum-packed storage up to 60 days, the 2-ACBs content remained unchanged in pork products irradiated at 2 and 5 kGy whereas a significant increase was observed in the pork products irradiated at 8 kGy.

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