LIPID OXIDATION AND 2-ALKYLCYCLOBUTANONESIN IRRADIATED TRADITIONAL PORK PRODUCTS

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Background

Irradiation is the treatment of food involving their exposure, either packaged or in bulk, to carefully controlled amount of ionizing radiation. Irradiation is considered a highly effective processing technology to achieve many technical objectives. Irradiation can kill harmful bacteria and other organisms in meat, poultry and seafood, disinfest spices, extend shelf-life of fresh fruits and vegetables killing insect eggs and larvae and control sprouting of tubers and bulbs such as potatoes and onions.
Similar to other food processes, irradiation has also technical and economic limitations that prevent its use on all foods under all circumstances. Low-dose irradiation treatments do not cause noticeable decreases in the nutritional quality of food, but the change in nutritional values depends on a number of factors: radiation dose, type of food, packaging and processing conditions such as temperature and oxygen exposure during irradiation and storage time (Crawford and Ruff, 1996). Irradiation causes certain chemical changes in food. Irradiation increased lipid oxidation in aerobically packaged meat and developed off-flavours (Ahn, *et al*., 2001b; Patterson and Stevenson, 1995).
Irradiation of fat-containing food generates also a family of molecules, namely 2-alkylcyclobutanones (2-ACB), that results from the radiation induced breakage of triglycerides. They are the only chemical compounds whose formation is thought to be specific for irradiation treatment of foodstuffs (Crone *et al*., 1992, 1993) and, for this reason they are useful for investigation of irradiated lipid-containing foods. These compounds contain the same number of carbon atoms as their precursor fatty acid and an alkyl chain of carbon atoms is attached to ring position 2. Due to the potential effects on human health the more investigated cyclobutanones are 2-dodecylcyclobutanone (2-DCB), 2-tetradecylcyclobutanone (2-TDCB) and 2-(5'-tetradecenyl) cyclobutanone (2-TdecCB). Their precursor fatty acids are listed in Table 1.
The Joint Expert Committee on the Wholesomeness of Irradiated Food of F.A.O., I.A.E.A. and W.H.O. concluded that irradiation of food up to an overall average dose of 10 kGy results in no toxicological hazard to humans. These organizations stated that irradiated foods are safe and nutritious (Joint F.A.O/W.H.O./I.A.E.A Expert Committee, 1981). Although irradiated foods have many known beneficial properties, the effect of long-term consumption of irradiated foods remains unknown. Lack of critical studies addressing the impact of long-term exposure to irradiated foods on human chronic diseases, such as cardiovascular disease, arthritis or various cancers, is considered a problem (Rao, 2003). Considering that some researchers underlined the problem about a potential toxicity of 2-ACB it is very important meanwhile to collect data concerning a wide variety of foods, wishing more investigations of this aspect.

Objectives

The aim of this investigation was to evaluate chemical effects of irradiation used as decontamination treatment on some typical Italian pork products. The research was focused in particular on the collection of preliminary data on the lipid oxidation and the formation of 2-ACB.

Materials and methods

The traditional Italian processed pork products investigated were: salame Milano, coppa and pancetta. Salame Milano is a typical minced, dry cured and fermented pork product; coppa is made up by the entire neck muscle, deboned, cured and afterwards matured in natural casing; pancetta is made up by adipose tissue of the ventral region of the pork and it looks layered alternatively fat and lean.
The products at the end of maturation were portioned and packed under vacuum in transparent plastic bags. For each kind of product the following groups were created: the control group (*Control*, n=5), the group irradiated at 2 kGy (*2kGy*, n=5) and the group irradiated at 5 kGy (*5kGy*, n=5). The treatment was performed using electron beam on refrigerated samples placed on cardboard boxes.

Proximate composition (AOAC, 1990) and fatty acid composition of total lipids (Zanardi et al., 2000) were determined on samples of *Control* groups. The samples belonging to *Control*, *2kGy* and *5kGy* groups were submitted to the following determinations:
- 2-thiobarbituric acid reactive substances test (TBARS test) as described by Tarladgis and Watts (1960), modified by Novelli et al. (1998). The procedure was based on the distillation of malondialdehyde and spectrophotometric determination at 534nm of the product of reaction between the distillate and 2-thiobarbituric acid;
- alkylcyclobutanones determination according the European Standard EN 1785: "Detection of irradiated food containing fat–Gas-chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones".
Among the cyclobutanones, the 2-DCB, the 2-TDCB and the 2-TdecCB were considered in this investigation. The procedure involved a first step of lipid extraction, a second step of purification and concentration of alkylcyclobutanones and their analysis by gas chromatography-mass spectrometry (GC-MS). 12 g of well homogenized sample were mixed with 12 g of anhydrous sodium sulphate in a thimble placed in a Soxhlet extractor and the lipid was extracted by refluxing 160 ml of petroleum ether for a minimum of 6 hours. An aliquot of 200 mg of fat was then dissolved by 1 ml of internal standard (0.5 µg/ml of 2-cyclohexylcyclohexanone in n-heptane). It was applied to the column of 30 g of inactivated Florisil (PR 60/100 mesh, Supelco) and eluted with 150 ml of hexane followed by 150 ml of 1% diethylether in n-hexane. This latter fraction was collected separately, concentrated using a rotary vacuum evaporator and transferred in a test tube. The sample was further concentrated to dryness under a stream of nitrogen and resuspended immediately in 500 µl of n-heptane. GC-MS analysis was carried out on a gas chromatograph 6890N (Agilent Technologies) coupled with a Mass Selective Detector 5973 Network (Agilent Technologies). The column was a DB-5 (30mx0.25 i.d., 0.25 µm film thickness, Supelco). Mass spectrometric analysis was performed by electron impact mode and positive ions. Selected ion monitoring (SIM) was set for 2-DCB and 2-TDCB for ions m/z 98 and 112 and for 2-TdecCB for ions m/z 67, 81, 98 and 109. The retention time and ion ratio of the signals were confirmed to correspond to those of standards (Fluka and Alfa Aesar for 2-cyclohexylcyclohexanone). Quantitative determination of 2-ACB was performed by using the peak areas of ion m/z 98 because common to all compounds. Their concentration was reported as µg/g of sample. The limit of quantification (LOQ) was 0.01 µg/ml for 2-DCB and for 2-TDCB and 0.2 µg/ml for 2-TdecCB.
Analysis of variance (ANOVA) was carried out on the data and the Scheffé's test ($p=0.05$) was used for the evaluation of significant differences (SPSS 11.5 for Windows). Not detected values of 2-ACB, because under the LOQ, were considered as 0.

Results and discussion

General chemical parameters fall in a normal range for products of such a type (data not shown). Data on fatty acid composition of total fat are not reported because not complete.
The data of TBARS values are shown in Table 2. The lowest mean values were found in coppa and they ranged between 0.083 and 0.088 mgMDA/kg. Not significant differences were found between *Control* and treated groups. The highest concentration of TBARS has been found in pancetta. The samples irradiated at 2 kGy reached, indeed, average value of 0.311 mgMDA/kg whereas *Control* and *5kGy* groups scored 0.187 and 0.130 mgMDA/kg respectively. Because of the relatively high standard deviation, however, no significant effect can be attributed to the irradiation treatment. In Salame Milano the samples of *Control* group showed higher values (0.230 mgMDA/kg) compared to *2kGy* (0.135 mgMDA/kg) and *5kGy* (0.124 mgMDA/kg) groups. Despite the significance resulting by statistics analysis, the TBARS values can be considered similar also in the Salame Milano. The high variability of TBARS values among meat products, indeed, has been widely recorded in previous studies and seems to be linked to an intrinsic variability of raw meat. All the results, however, were lower than the suggested threshold for the appearance of rancidity off-flavours in fresh pork (0.5 mgMDA/kg) (Lanari *et al*., 1995). TBARS values were in line with the results of previous investigations (Novelli et al., 1998; Zanardi et al., 2000). The latter mentioned study reports, for example, TBARS values of Salame Milano and coppa around 0.280 and 0,110 mgMDA/kg, respectively, confirming the lower values in coppa. These values proved that, in this case, the irradiation does not accelerate lipid oxidation. This result could be explained by the vacuum packaging adopted in this research. Oxygen has a catalytic effect on irradiation-induced lipid oxidation (Lambert *et al.*, 1992). As has been suggested by some authors, the exclusion of oxygen should help reduce the extent of oxidation of fatty acid in vacuum packed cooked pork sausages compared to the same products packed in aerobic packaging

irradiated at 5kGy (Jo et al., 2003). The same effect of packaging has been observed on cholesterol oxidation in cooked pork (Ahn et al., 2001a). Moreover, the irradiation treatment was carried out on refrigerated pork products. Nawar and Balboni (1970) reported that the amounts of radiolytic products formed on irradiation are sensitive to the temperature at which the irradiation take place.
The data of 2-ACB content in salame Milano, pancetta and coppa are partially showed in Table 3. The three 2-ACB considered in this investigation were under the limit of detection (LOD) in all the pork products of *Control* groups. The irradiation dose 2kGy induced the formation of 2-ACB in all the three types of products. In particular the 2-DCB was observed in a range of values between 0.019 and 0.054 μg/g and the 2-TDCB between 0.029 and 0.039 μg/g in the samples of *2kGy* groups. The irradiation dose 5kGy did not further increase the content of both 2-ACB in salame Milano and coppa whereas induced a significant increase ($p=0.05$) of both 2-DCB and 2-TDCB in pancetta in which 2-DCB reached 0.123 μg/g and 2-TDCB 0.102 μg/g. When these concentrations of 2-ACB were plotted against irradiation doses, a linear response was observed both for 2-DCB and for 2-TDCB (Figure 1). These concentrations resulted also statistically higher ($p=0.05$) than those of 2-DCB and 2-TDCB in salame Milano and in coppa. Probably the difference among pancetta, salame Milano and coppa was due to the different fat content. The average percentage of fat in pancetta was, indeed, about 40%, whereas in salame Milano and in coppa was about 30%.
In Table 3, the values of 2-TdecCB were not included, because it was found occasionally. In all the samples of *Control* groups, in these ones irradiated at 2 kGy and in samples of coppa irradiated at 5 kGy the 2-TdecCB was not detected. These results could be explained by the high limit of quantification (0.2 μg/ml) due probably to the much greater fragmentation of 2-TdecCB in the ion source of the MS. 2-TdecCB was found only in salame Milano and in pancetta irradiated at 5 kGy. The average values were 0.355±0.224 and 0.969±0.422 μg/g respectively. These latter values were higher than the concentrations of both 2-DCB and 2-TDCB at the same dose of irradiation. This result was in line with Park *et al.* (2001) who detected the same 2-ACB in irradiated pork: 2-TdecCB showed the highest content for all irradiation treatments in the range of doses between 0.5 and 10 kGy. Compared to the data reported by the same author, the average content of 2-ACB on fat basis of the present study was lower. Due the absence of data on the use of irradiation of traditional pork products, the explanation of the results of the present investigation could be attributed also for 2-ACB to the packaging conditions. The absence of oxygen could reduce the induced radical species in the matrix.

Conclusions

The present study is a collection of preliminary data on the lipid oxidation and formation of 2-alkylcyclobutanones in some traditional pork products treated by ionising radiation as a decontamination process. The extent of lipid oxidation was not significantly influenced by ionising radiation at 2 and 5 kGy irradiation doses. 2-alkylcyclobutanones have been confirmed to be useful markers of identification of irradiated fat-containing food. 2-DCB and 2-TDCB can provide useful information to distinguish between treated and not irradiated food. 2-TdecCB could be used as an additional marker. Its high limit of quantification can not guarantee the distinction between treated and not irradiated food.
The conditions used during the treatment (vacuum packaging and refrigeration) seem influence both lipid oxidation and formation of 2-alkylcyclobutanones. The packaging can be considered a critical factor affecting the chemical quality of irradiated pork products.

Acknowledgments

The authors gratefully acknowledge the cooperation and the assistance of Dr. Alberto Butturini and Dr. Rita Tagliazucchi of Villani S.p.A. (Castelnuovo Rangone, Modena, Italy)

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Table 1: 2-ACB considered in this investigation and their precursor fatty acid.

Precursor fatty acid	2-ACB
Palmitic acid ($C_{16:0}$)	2-dodecylcyclobutanone
Oleic acid ($C_{18:1}$)	2-(5'-tetradecenyl)cyclobutanone
Stearic acid ($C_{18:0}$)	2-tetradecylcyclobutanone

Table 2: TBARS (mg malondialdehyde/kg) values (mean ± standard deviation) of salame Milano, pancetta and coppa.

Pork product	Control	2 kGy	5 kGy
Salame Milano	0.230 ± 0.069^{a}	0.135 ± 0.021^{b}	$0.124 \pm 0{,}007^{b}$
Pancetta	0.187 ± 0.080^{a}	0.311 ± 0.191^{a}	0.130 ± 0.027^{a}
Coppa	0.085 ± 0.009^{a}	0.088 ± 0.014^{a}	0.083 ± 0.020^{a}

[a,b]: different letters on the same raw stand for significant differences, p=0.05, Scheffé's test

Table 3: 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TDCB) content (µg 2-ACB/g) (mean ± standard deviation) of salame Milano, pancetta and coppa

Product	2-ACB	Control	2 kGy	5 kGy
Salame Milano	2-DCB	N.D.[a]	0.019 ± 0.008^{b}	$0.030 \pm 0.005^{b,e}$
	2-TDCB	N.D.[a]	0.029 ± 0.030^{ab}	$0.046 \pm 0.015^{b,e}$
Pancetta	2-DCB	N.D.[a]	0.054 ± 0.006^{b}	$0.123 \pm 0.029^{c,d}$
	2-TDCB	N.D.[a]	0.037 ± 0.005^{b}	$0.102 \pm 0.018^{c,d}$
Coppa	2-DCB	N.D.[a]	0.042 ± 0.011^{b}	$0.048 \pm 0.019^{b,e}$
	2-TDCB	N.D.[a]	0.039 ± 0.012^{b}	$0.061 \pm 0.027^{b,e}$

[a,b,c,]: different letters on the same raw stand for significant differences, p=0.05P<=0.05, Scheffé's test
[d,e]: different letters on the same column stand for significant differences, p=0.05, Scheffé's test
N.D.=not detected

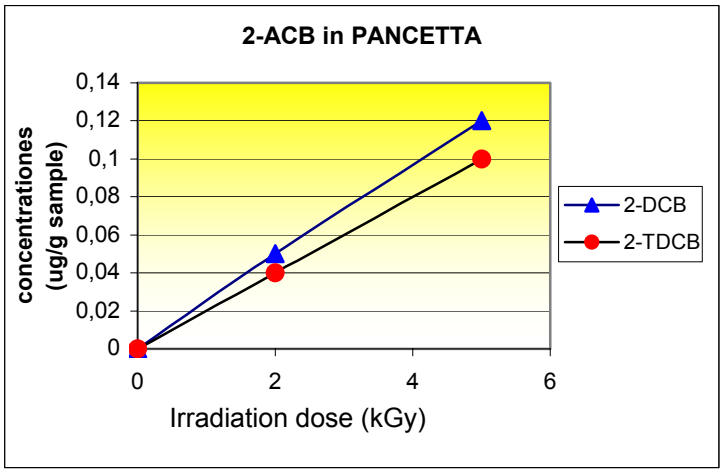

Figure 1: Effect of irradiation dose on 2-DCB and 2-TDCB produced in irradiated pancetta.