

Effects of gamma irradiation or high hydrostatic pressure on cholesterol oxidation and sensorial quality in mechanically deboned turkey meat and chicken liver

V.K. LEBOVICS^{1*}, Ö. GAÁL¹, A. LUGASI¹, L. MÉSZÁROS¹, É. ANDRÁSSY² and J. FARKAS²

¹National Institute for Food Safety and Nutrition, Department of Food Chemistry, 1097 Budapest, Gyáli út 3/a, Hungary

²Central Food Research Institute, 1022 Budapest, Herman Ottó út 15, Hungary

*lebovics@okk.antsz.hu

Keywords: food processing; ionising radiation; high-hydrostatic pressure; cholesterol oxides; thiobarbituric acid-reactive substances; mechanically deboned poultry meat; chicken-liver; food storage

Pasteurising effect of 2 kGy radiation dose or 200 MPa hydrostatic pressure for 20 min in non-frozen mechanically deboned turkey meat (MDM) was achieved without increase of cholesterol oxidation products (COPs) and increases of TBARs values (thiobarbituric acid-reactive substances) during 15 days of chilled storage following the treatments, while untreated samples were spoiled. In frozen MDM, 7 kGy was equivalent to 400 MPa for 20 min in respect of improved microbiological safety, however, accelerated oxidation of cholesterol and lipids as well as organoleptic changes were observed. Due to considerable increase in the amount of COPs in untreated samples during frozen storage, the same level of oxysterols in control as well as in treated samples was found after 8 months of frozen storage. Similar changes in TBARs values were observed. In experiments with non-frozen samples, addition of antioxidants, such as thyme-oil or α -tocopherol + ascorbic acid inhibited significantly the oxidative changes of cholesterol and lipids during 3 kGy or 400 MPa treatments. The pasteurising effects of 300 MPa treatments and 2 kGy, and that of oxidative changes were tested on non-frozen chicken liver. Although favourable microbiological effects were achieved, 2 kGy irradiation induced off-odour while the high- hydrostatic pressure (HHP) treatment caused considerable discolouration and a slight off-odour.

Introduction

The problem of contamination of poultry with pathogenic bacteria is greatly aggravated in case of such by-products of poultry processing as liver or mechanically recovered meat. Ionising radiation or high hydrostatic pressure (HHP) treatments are efficient non-thermal tools to destroy non-spore-forming bacteria (Farkas, 2004; Hendrickx and Knorr, 2001). In principle, both technologies offer opportunity to produce foods of high sensory and nutritional quality with extended shelf-life without use of additives. However, development of proper decontamination technologies requires prevention of unwanted side effects on quality of the decontaminated products. Our earlier work (Lebovics *et al.*, 2002) and work in the literature (Nam and Ahn, 2003; Gomes and Silva, 2003) showed that γ -irradiation accelerates the formation of cholesterol oxidation products (COPs) and increases the TBARs values (thiobarbituric acid-reactive substances), but no data were available on the fate of sterols, and very few on lipid changes in poultry meat during the application of the emerging HHP technology (Dissing *et al.*, 1997).

Certain cholesterol oxidation compounds have potentially undesirable biological activities such as atherogenicity, atherosclerosis, cytotoxicity, mutagenicity, carcinogenicity, furthermore may cause cell membrane damage and can inhibit the cholesterol biosynthesis. Current knowledge of cholesterol oxidation and its biological effects has been reviewed in detail by Guardiola *et al.*, (2002). Cholesterol oxides are present in certain foodstuffs in concentration of a few mg/kg.

Cholesterol and lipid oxidation are affected by various factors, such as light, heat, radiation, oxygen, moisture, pH, certain pro-oxidising agents, processing procedures, storage conditions.

In our present studies the effects of γ -irradiation and HHP on the microbiological status as well as on oxidation of cholesterol and other lipids were compared. The efficiency of natural antioxidants in suppressing the undesirable oxidative changes during the treatments in fresh and chilled or frozen

stored samples were also studied. Similar, preliminary studies were performed also with chicken liver.

Materials and methods

RAW MATERIALS AND PACKAGING

Numerous 25-40 g portions of commercial mechanically deboned turkey meat (MDM) or chicken liver were packaged into small plastic pouches formed from "Multibarrier 4" PE-PA laminated foil of 100 μm thickness (produced by WIPAK, Finland), oxygen transmittance: 5 mL/m²/24h.atm at 23°C and 50% relative humidity, and sealed without evacuation by a MULTIVAC Mod. 300 machine (Haggenmüller, Walfertschwerden, Germany).

TREATMENTS

Ionising radiation

Mechanically deboned turkey meat

In one part of the experiments the MDM samples were treated with a dose of 7 kGy in a Co-60 self-shielded radiation source, type RH- γ -30 at a dose rate of 3,9 kGy/h in frozen state, than stored together with respective un-irradiated samples at -20°C for 8 months.

In two other experiments the samples were treated with a dosage level of 2 kGy and 3 kGy, respectively, at approx. 5°C in the presence of antioxidant additives, then stored together with respective un-irradiated samples at 4°C for 15 days.

Chicken liver

The irradiation was performed with dose of 2 kGy at approx. 5°C.

High hydrostatic pressure treatment

Isostatic pressurization was performed in a hydrostatic plunger press of 0.3 L chamber volume, type „FoodLab 900" (Stansted Fluid Power, Stansted, U.K.) while their temperature was kept between 10 and 20°C using the cooling system Haake C40-FB. Ethanol containing 15% castor oil was applied as pressurizing medium.

The MDM samples were subjected to high pressure treatments as follows: a) 200 MPa for 20 min, then stored at 4°C for 15 days; b) 400 MPa for 20 min, then stored for 8 months at -18°C, and c) 400 MPa for 20 min in the presence of antioxidants, then stored for 3 months at -18°C. The chicken liver was treated at 300 MPa for 20 min. Both pressure build-up and pressure release took about 1 min.

Addition of antioxidants

For assessing the effects of antioxidants, before packaging and treatments the MDM was divided into three experimental batches: 1) without antioxidants; 2) with addition of 250 mg thyme-oil/100 g, and 3) with addition of 12 mg α -tocopherol plus 25 mg ascorbic acid/100 g sample.

ANALYSES

Microbiological status

Total aerobic viable cell counts (TVCs) in TGE plates (Merck 1.05463) and Enterobacteriaceae counts on VRBG agar (Oxoid CM485) were estimated, incubation at 30°C for 48 hours.

Measurement of thiobarbituric acid-reactive substances (TBARs-values)

The TBARs values were estimated according to Ramanathan and Das (1993) and expressed as malondialdehyde.

Determination of cholesterol oxides

Approx. 50 g of meat samples or 100 g of chicken liver were extracted with chloroform/methanol (2:1 v,v) according to Folch *et al.*, (1957). After evaporation of solvents the lipid extract was dissolved in 200 ml 1 M potassium hydroxide in methanol and saponified by refluxing for 60 min under stream of nitrogen. 200 ml of distilled water were added to the saponified mixture and the non-saponified organic layer was extracted three times with 100 ml diisopropyl-ether (Fluka AG, puriss) and the combined fractions were washed three times with 200 ml distilled water and dried with anhydrous sodium sulphate (Missler *et al.*, 1985). The filtrate was evaporated to dryness under vacuum. The residue was dissolved in chloroform/methanol (2:1 v,v) to make a concentration of 0,1 g/ml (Lebovics *et al.*, 1992).

Separation and identification of cholesterol oxidation derivatives

The non saponifiable extract was analysed by HPTLC using pre-coated HPTLC Sil G 60F₂₅₄ thin-layer 10x10 cm glass plates, purchased from E. Merck (Darmstadt, Germany). The chromatoplates were developed twice in heptane-ethylacetate (1:1,v). The plates were examined under UV light (254 nm) and sprayed with 100 g litre⁻¹ CuSO₄•5H₂O dissolved in 85 ml litre⁻¹ phosphoric acid and heated at 110°C for several minutes for colour development.

The identity of spots was confirmed by comparison with authentic standards of known colour and R_F-values. (Lebovics *et al.*, 1992)

Quantitation

The spots of silicagel adsorbents containing oxysterols were scraped and eluted. The determination of oxysterols was performed by the test system 'cholesterol enzymatique PAP 250 ref 61225' based on the effect of cholesterol oxidase. (Lebovics *et al.*, 1996).

Results

COMPARISON OF 7KGY IRRADIATION AND 400 MPa TREATMENTS DURING 8 MONTH FROZEN-STORAGE

For the microbiological safety of frozen raw material higher doses of irradiation and HHP treatments were appropriate.

Microbiological status

The pasteurising effect of these treatments was successful and equivalent which is shown in Table 1.

Table 1 Total aerobic viable cell counts (TVCs) in mechanically deboned turkey meat (log CFUs/g) after 400 MPa and 7 kGy treatments and frozen storage.

	0 day	Frozen storage (-20°C)	
		4 months	8 months
Control	5•10 ⁴	3,5•10 ²	3•10 ²
400 MPa	< 1•10 ²	< 1•10 ²	< 1•10 ²
7 kGy	< 1•10 ²	< 1•10 ²	< 1•10 ²

Both radiation and high-pressure treatments reduced the TVC by more than 2 decimals and no further changes were observed during 8 months storage.

Sensory changes

Both treatments resulted in unfavourable off odour and brownish colorization.

Cholesterol oxidation products

Tables 2 and 3 present the results of COPs measurements in samples treated with higher dosages of non-thermal treatments

Table 2 Concentration of COPs (mg/kg) in untreated samples of mechanically deboned turkey meat and those treated with 400 MPa hydrostatic pressure.

COPs	CONTROL			400 MPa		
	0 day	4 months	8 months	0 day	4 months	8 months
cholestan-3 β ,5 α ,6 β -triol	nd	2,58	3,18	nd	3,74	4,22
7 α -OH	nd	1,80	2,08	1,66	3,53	4,42
7 β -OH	nd	2,84	3,84	1,32	3,75	4,46
7-keto	nd	nd	3,98	1,32	3,33	4,00
5 α ,6 α -epoxide	nd	nd	1,41	nd	2,91	2,68
Total COPs	nd	7,22	14,49	4,30	17,26	19,78

nd: < 0,7 mg/kg, total lipid content:17,7%, cholesterol content:707 mg/kg

7 α -OH: 7 α -hydroxycholesterol;

7 β -OH: 7 β -hydroxycholesterol;

7-keto:7-ketocholesterol;

5 α ,6 α -epoxide: cholesterol-5 α ,6 α epoxide

Table 3 Concentration of COPs (mg/kg) in untreated samples of mechanically deboned turkey meat and those treated with 7 kGy irradiation.

COPs	CONTROL			7 kGy		
	0 day	4 months	8 months	0 day	4 months	8 months
cholestan-3 β ,5 α ,6 β -triol	nd	2,58	3,18	nd	3,71	4,60
7 α -OH	nd	1,80	2,08	2,60	3,34	4,39
7 β -OH	nd	2,84	3,84	2,31	3,54	4,07
7-keto	nd	nd	3,98	1,83	4,39	4,14
5 α ,6 α -epoxide	nd	nd	1,41	1,23	3,25	3,23
Total COPs	nd	7,22	14,49	7,97	18,23	20,43

nd: < 0,7 mg/kg, total lipid content:17,7%, cholesterol content:707 mg/kg

Abbreviations as in Table 2.

There were no detectable amounts of COPs in the raw material at 0 day, but both treatments resulted in the formation of the following oxysterol products: cholestan-3 β ,5 α ,6 β -triol, 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, 7-ketocholesterol, cholesterol-5 α ,6 α -epoxide.

At day 0 the ionising radiation caused more significant oxidative changes than that of HHP treatment. During 4-month storage the levels of COPs increased three-fold as compared to the control samples. After 8-month storage the COPs concentration was equalized in all samples. Similar changes were observed in TBARs values (not shown).

COMPARISON OF 2 KGY IRRADIATION AND 200 MPA TREATMENTS DURING 15 DAY CHILLED-STORAGE

For the extended chilled storage of the freshly produced raw material, lower doses of treatments were applied.

Microbiological status

The pasteurising effect of these treatments was also successful and equivalent which are shown in the Table 4.

Table 4 Total aerobic viable cell counts (TVCs) in mechanically deboned turkey meat (log CFUs/g) after 200 MPa and 2 kGy treatments and storage.

	Chilled storage (+4°C)	
	0 day	15days
Control	$7 \cdot 10^3$	$8 \cdot 10^{7**}$
200 MPa	$8 \cdot 10^2$	$7 \cdot 10^2$
2 kGy	$8 \cdot 10^2$	$7 \cdot 10^2$

** The samples were spoiled

Both treatments reduced the TVC by 1 decimal at 0 day and no further changes were observed during 15 day storage at treated samples, but the untreated samples were spoiled.

Sensory changes

Neither of treatments resulted in unfavourable off odour and colour changes.

Cholesterol oxidation products

Table 5 presents the results of COPs measurements in samples treated with lower dosages of non-thermal treatments

Table 5 Concentration of total COPs (mg/kg) in untreated samples of mechanically deboned turkey meat and those treated by 200 MPa high hydrostatic pressure and 2 kGy irradiation, respectively, followed by refrigerated storage for 15 days.

	Control		200 MPa		2 kGy	
	0 day	15 day	0 day	15 day	0 day	15 day
Total COPs	0,18	X	0,19	0,20	0,19	0,67

x- the samples were spoiled

There were measurable initial levels of total COPs in the raw material, but neither of the treatments caused changes in the level of COPs at 0 day. Fifteen days chilled storage practically did not increase the oxidative changes of cholesterol and the low TBARs-values (0,035 MA eqv. mg/kg) at 0 day remained unchanged.

Both treatments were favourable regarding the negligible oxidative changes of cholesterol and other lipids. The microbiological status of the samples was satisfactory even during 15-day storage. Thus, both dosage levels would be suitable for practical application.

APPLICATION OF 3 KGY IRRADIATION IN THE PRESENCE OF ANTIOXIDANTS IN MECHANICALLY DEBONED TURKEY MEAT STORED CHILLED FOR 15 DAY

The aim of this experiment was to reduce the undesirable oxidative changes during the irradiation and storage.

Microbiological status

Radiation treatment reduced the TVC by 3.5 log units (from log CFU/g=4.75 to log CFU/g=1.20) and the Enterobacteriaceae count from log CFU/g=4.1 to below the detection limit (log CFU/g<1.0) as estimated directly after treatment (0 day) in the antioxidant-free samples. In the thyme oil-containing samples the residual TVC at 0 day was log CFU/g=1.6 whereas log CFU/g=2.0 in the tocopherol-ascorbic acid containing samples. During 15 days of chilled storage, un-irradiated samples reached a spoilage level of TVCs (log CFU/g= 8.75).

Cholesterol oxidation and TBARs

Results of COPs and TBARs measurements are presented in Tables 6 and 7, respectively.

Table 6 Concentration of COPs (mg/kg) in un-irradiated and gamma-irradiated mechanically deboned turkey meat (average from duplicated samples in two parallel experiments).

COPs	Day 0 (un-irradiated)			Day 0 (3 kGy)			Day 15 at 4 °C (3 kGy)		
	C	AO	T	C	AO	T	C	AO	T
7 α -OH	2,04	1,11	0,89	3,06	1,40	1,34	3,63	1,73	1,78
7 β -OH	2,12	1,04	0,79	2,02	1,58	0,92	2,14	1,64	2,54
7-keto	2,50	Nd	nd	2,70	nd	nd	2,39	1,67	0,97
5 α ,6 α -ep	2,30	Nd	nd	2,28	nd	nd	2,37	nd	nd
Total	8,96	2,15	1,68	10,06	2,98	2,26	10,53	5,04	5,29

Cholesterol content: 668,6 mg/kg

Abbreviations as in TABLE 2.

C: antioxidant-free control; AO: α -tocopherol+ascorbic acid; T: thyme oil.

nd < 0,30 mg/kg

Abbreviations: as in Table 3.

Table 7 TBARs (MAD mg/kg) in un-irradiated and gamma irradiated mechanically deboned turkey meat.

Day 0 (un-irradiated)			Day 0 (3 kGy)			Day 15 at 4 °C (3 kGy)		
C	AO	T	C	AO	T	C	AO	T
4.42	1.64	1.35	7.43	2.31	1.61	5.35	2.47	2.06

Abbreviations as in Table 6.

Several COPs, such as 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, and cholesterol-5 α ,6 α -epoxide were found even in the un-irradiated samples before irradiation and storage. Gamma irradiation enhanced the cholesterol and lipid oxidation (causing approx. 20% increase in the COPs level, and 50% increase in TBARs values at 0 day in the antioxidant-free samples. 15 day storage did not cause an increase in COPs level in irradiated samples without addition of antioxidants. The presence of antioxidants reduced significantly both the amounts of COPs and TBARs values.

APPLICATION OF 400 MPa HHP TREATMENT IN THE PRESENCE OF ANTIOXIDANTS IN MECHANICALLY DEBONED TURKEY MEAT STORED FROZEN FOR 3 MONTHS

Table 8 Concentration of COPs (mg/kg) in untreated and 400 MPa high pressure treated mechanically deboned turkey meat (average from duplicated samples in two parallel experiments) stored frozen for 3 months.

COPs	Day 0 (untreated)			Day 0 (400 MPa)			3 months(untreated)			3 months(400 MPa)		
	C	AO	T	C	AO	T	C	AO	T	C	AO	T
7 α -OH	0,66	0,32	0,45	0,80	0,57	0,46	0,68	0,66	0,46	2,32	0,83	0,44
7 β -OH	0,60	0,32	0,47	0,63	0,49	0,37	0,59	0,61	0,38	1,70	0,77	0,42
7-keto	0,62	nd	0,45	0,97	nd	0,57	0,64	0,51	0,46	0,86	0,88	0,63
5 α ,6 α -epoxid	nd	nd	Nd	nd	nd	nd	0,60	0,83	0,40	0,95	0,35	0,28
Total	1,88	0,64	1,37	2,40	1,06	1,40	2,51	2,61	1,70	5,83	2,83	1,77

Cholesterol: 488 mg/kg, total lipid: 90,7 g/kg

Abbreviations as in TABLE 2.

C: antioxidant-free control; AO: α -tocopherol+ascorbic acid; T: thyme oil.

nd < 0,30 mg/kg

The addition of thyme-oil caused favourable changes in colour, the slightly brownish colour become pink-reddish, characteristic to fresh product. The antioxidants in this experiment were highly effective in preventing oxidative changes similarly to that observed in the case of application

of 3 kGy irradiation. However, the 400 MPa treatment caused slight increases in the level of COPs, but tocopherol-ascorbic acid reduced the oxidation of cholesterol by 60%, and the thyme-oil reduced by 30-40%. During 3-month storage the thyme-oil decreased the formation of COPs with 30% at the control samples and with 70% at the treated samples.

COMPARISON OF 2 KGy IRRADIATION AND 300 MPa HHP TREATMENT OF THE CHICKEN LIVER

This test material was chosen because of its high lipid- and cholesterol content. There were some preliminary experiments to choose the equivalent pasteurizing doses in chicken liver. During these experiments it was found that 2 kGy and 300 MPa for 20 min resulted in equivalent reduction in the viable cell count.

Microbiological status

Both treatments resulted in decrease of 1 order of magnitude in total viable cell count, as it is shown in Table 9.

Table 9 Total aerobic viable cell counts (TVCs) in chicken liver (log CFU/g) at 2 kGy and 300 MPa for 20 min treatments.

	Control	2 kGy	300 MPa
TVCs	3,76	2,45	2,55
Enterobacteriaceae	4,58	<1,00	<1,00

Table 10 Concentration of total COPs (mg/kg)* and TBARs (MAD mg/kg) in untreated samples of chicken liver and those treated 2 kGy irradiation and 300 MPa high hydrostatic pressure, respectively.

COPs	Control	2 kGy	300 MPa
cholestan-3 β ,5 α ,6 β -triol	0,84	0,81	0,79
7 α -OH	0,96	0,95	1,07
7 β -OH	0,20	0,54	0,66
25-OH	1,30	1,60	0,98
Total COPs	3,30	3,90	3,50
TBARs	1,08	4,00	4.20

*the data represent the average of 2 parallel measurements, dry weight: 29,8%, total fat:5,8%, cholesterol:1050 mg/kg

Abbreviations as in TABLE 2., 25-OH: 25-hydroxycholesterol

Although the oxidation of cholesterol and other lipids was negligible during these treatments, and the microbiological effect was satisfactory as well, there were significant changes in colour and odour. 2 kGy caused a characteristic off odour.

Discussion and conclusions

Irradiation as well as high hydrostatic pressure can accelerate lipid oxidation and produces off odour in meat. Turkey meat is rather susceptible to oxidation due to its relatively large content of membrane-associated phospholipids in combination with a low endogenous level of tocopherols (Dissing *et al.*, 1997). The pasteurizing doses of 7 kGy gamma radiation as well as 400 MPa high hydrostatic pressure improved the microbiological quality of mechanically deboned frozen turkey meat, however, they enhanced under aerobic packaging condition the concentrations of COPs and TBARs. In the same time, unfavourable sensory changes occurred. The COPs formed in the MDM samples were mainly 7 α -hydroxycholesterol, 7 β -hydroxycholesterol (early and sensitive indicators of oxidation of cholesterol), 7-ketocholesterol and cholesterol-5 α ,6 α -epoxide. All of these compounds may have significant health implications. 2 kGy gamma radiation and 200 MPa HHP did not result in oxidative changes of cholesterol and in other lipids in mechanically deboned non-frozen turkey meat, but increased the microbiological stability during chilled storage without any sensory changes. Natural antioxidants tested were highly effective in suppressing oxidative

changes of cholesterol and other lipids as well as off-odour production during the application of 3 kGy irradiation or 400 MPa HHP treatments. These observations confirmed our earlier results (Lebovics *et al.*, 2002) and others' literature data (Chen *et al.*, 1999). The mechanism of the decrease of the concentrations of preformed oxidation products due to the addition of antioxidants is not clear yet and will be investigated. The reduction/destruction of the intermediate peroxides might be involved in these processes. 2 kGy or 300 MPa did not cause significant chemical changes of lipids, but unfavourably influenced the sensory properties.

2 kGy caused a characteristic off-odour and 300 MPa induced yellow discolouration and soft texture in the brown livers of chicken. Thus, the application potential of ionising radiation or HHP treatment seem to be limited in the case of chicken liver.

Information on the effects of non-thermal decontamination treatments on cholesterol oxidation in relevant foods are still relatively limited, and are mostly focused on radiation processing (Lebovics *et al.*, 1992, 1994; Hwang and Maerker, 1993; Ahn *et al.*, 2001; Nam *et al.*, 2001). All these studies showed that the presence of the oxygen, which depends on packaging and treatment conditions, is one of the most important factors in the formation of COPs and lipid oxidation. Further experiments are needed to check the possible practical applications of these findings to avoid the adverse effects of γ -irradiation or high hydrostatic treatment in order to produce safer foods.

Acknowledgements

This work was financially supported by the Hungarian National Research Funds (OTKA, grants no. T029567 and T032715).

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