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Effect of Grilling, Roasting, and Cooking on the Natural Hexachlorobenzene Content of Ovine Meat

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Processed foods play an important role in the current human diet. Most meat and vegetables for consumption are subjected to different operations during preservation and preparation. The procedures used may actually reduce or remove pesticide residues that can affect human health. However, it is not easy to establish the influence of different treatments. It seems that pesticide residues show a different behaviour depending on their artificial or natural presence in foods. Additionally, some technological processes, such as homogenising of milk plus certain heating treatments, can increase the extractability of pesticides, which result in an apparent higher residue content after these operations.

Since the detection of high hexachlorobenzene (HCB) levels in human adipose tissue and human milk samples in the Spanish population by Polo et al. (1978) and Pozo et al. (1979), several papers have been published in this country reporting high HCB levels. The 1st National Conference on HCB (Anonymous 1990) concluded that the level of HCB in Spanish foods and samples of human origin was one of the highest in the world. Ferrer et al. (1992) report the presence of HCB residues in all the samples in a study involving 168 human adipose tissue samples.

HCB causes an illness known as "Turkish porphyria" that is characterised by skin lesions due to photosensitization and neurological problems, such as insensitivity of the extremities and Parkinson's syndrome. Concon (1988) reported the carcinogenic effect of HCB in experimental animals and the IV Annual Report on Carcinogens (1985) considered HCB as a potential human carcinogen (Merck Manual 1989).

Recently Ariño (1991) observed that cooking and ripening have no influence on the residual amount of HCB in two kinds of pork sausage,

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but processing of Spanish dry-cured ham for 6 months removed 42% of the HCB residues ($p<0.001$). Van Renterghem (1976) observed a significant reduction ($p<0.05$) of the initial HCB content in pasteurised milk (73°C/15 sec). On the other hand, both sterilisation (118°C/10 min) and ultra high temperature (150°C/2,4 sec) produced a significant increase of HCB content due to the homogenization of fat globule. More recently, Garrido Fernández (1990) reported a significant reduction ($p<0.05$) of HCB content by 74% in spiked milk samples (3 ppm of HCB) subjected to sterilisation (115°C/15 min).

We have not found any studies in the literature concerning the influence of cooking treatments on natural HCB contents of meat. In the present study we have investigated HCB contamination in ovine meat samples that were subjected to three different culinary treatments to evaluate sanitary risks derived from its usual consumption.

MATERIALS AND METHODS

The present study was conducted with 75 meat samples belonging to three commercial pieces each consisting of 25 ovine carcasses (chop, leg and lap), both raw and prepared by grill, roasting, and pressure cooking.

1) Chop samples (cross section of the *longissimus dorsi*) (n=25) consisting of four or five medial chops were grilled without oil or dressing. The gridiron was preheated to the maximum temperature as indicated by a thermostat. Then, samples were heated for 5 min at 85°C (internal temperature measured by a penetration thermometer) and cooled at room temperature before analysis. When it was necessary, the sample was kept at 4°C until analysed.

2) Leg samples (cross section at the *corpus ossis femoris*) (n=25) roasted for 45 min in a conventional electric oven preheated to 220±2°C. Samples reached an internal temperature of 100±1°C as indicated by a penetration thermometer.

3) Lap samples (200 g weight) (n=25) composed of abdominal muscles subjected to cooking treatment in a pressure cooker. Samples were laid in a metallic basket after addition of distiled water previously tested for pesticide residues. For this treatment the sample was subjected to 130°C for 10 min.

The samples were analysed before and after being prepared by its respective culinary treatment. Extraction and cleanup of residues were performed using the method by Telling et al. (1977) as previously described (Conchello 1991). Briefly, the method is based on a sample homogenization with acetone-hexane (1:4) and cleanup of the fat

extract on a 22-g single column of activated alumina eluted with 150 mL of hexane. The eluate is concentrated to 2 mL and analysed by GLC-ECD.

The fat content was determined gravimetrically by transferring an aliquot of hexane extract to a pre-weighed Erlenmeyer flask and then evaporating the solvent.

The alumina activity must be determined experimentally by the addition of predetermined amounts of water in order to retain 0.62±0.02 g of animal fat.

ECD-GLC analysis was performed with two chromatographs, Hewlett-Packard model 5890A and 5890 Series II, equipped with a packed column (6-ft, 1/4-in. od, 3-mm id) 1.5% SP-2250/1.95% SP-2401. Two additional different packed columns were used for confirmatory analysis: 4% SE-30/6% QF1 and 5% QF1. Operating parameters are given in Table 1. Detection limit of HCB was 4 μg/kg on a fat basis.

Table 1. Operation parameters of the columns employed

Parameter	A	B	C
T (°C) Column	200	195	195
T (°C) Inlet	250	250	250
T(°C) Detector	300	300	300
Carrier gas	Ar/CH_4 or N_2	N_2	N_2
Flow (mL/min)	50 - 52	30	42

A: Column 1.5 % SP-2250 + 1.95 % SP-2401
B: Column 5 % QF-1
C: Column 4 % SE-30 + 6% SP-2401

As it was impossible to obtain samples without HCB residues and due to the absence of international reference material, a recovery study was performed at the cleanup step using 2 mL of a hexane solution at two HCB levels (1 and 10 μg/L). Percentages of recovery are calculated by dividing the mean area value of the concentrated eluate corresponding to four assays by those of the pattern solution multiplied by hundred. Both levels showed good recoveries, 85.6%±3.5 (% C.V.; n=4) at 1 ppb and 91.8%±3.6 (% C.V.; n=4) at 10 ppb.

RESULTS AND DISCUSSION

Table 2 shows the results in μg/kg fat basis obtained in both raw and treated chop, leg and lap samples, as well as the variation of the HCB content.

Table 2. Cooking treatment influence on the HCB content. Wilcoxon's test

Treatment	n	%	Mean	Max.	% Reduction	WILCOXON
Raw chop	25	100	48.9	180	17.7	**
Grilled chop	25	100	40.2	172		
Raw leg	25	100	47.4	183	17.9	**
Roasted leg	25	100	39.0	182		
Raw lap	25	100	50.6	172	18.5	**
Cooked lap	25	100	41.2	202		

n. s.: no significant
** $p < 0.01$ Signification level

HCB was present in all the raw samples showing a mean value of 48.9 ppb ± 8.0 (std. dev.) in chop samples, 47.4 ppb ± 7.8 in leg samples, and 50.6 ppb ± 8.2 in lap samples. The average contamination of the 75 raw samples was 49.0 ppb ± 7.9. When comparing the initial HCB contamination to the contamination after all treatments, a decrease of HCB level can be observed, whereas the detection percentages remained unchanged at 100%, i.e., all samples contained residues.

The mean concentration went down by 17.7%, from 48.9 ppb to 40.2 ppb in chop samples after grill treatment. In the case of leg samples, losses averaged 17.9%, from 47.4 ppb to 39.0 ppb. Cooking treatment of lap samples produced the highest losses; residues decreased from 50.6 ppb to 41.2 ppb, or 18.5%. Wilcoxon test showed a significant reduction ($p<0.01$) for all of the three cooking treatments studied.

HCB concentration showed an increase in some of the individual samples after treatment. Increase and decrease frequencies for each treatment (Table 3) did not show significant differences as compared by χ^2 test ($p>0.05$). As many as 11 out of 75 samples (14.7%) showed higher HCB content after heating treatment. A significant decrease was demonstrated in most of the samples, as shown in Table 3.

Our data are different from those reported by Ariño (1991) where a great percentage of pork sausage samples maintained the same content after cooking treatment or even had an increase in HCB levels. This can be explained by the lower temperature used in the processing (80-82°C) and the impermeability of the plastic casing which prevents drip and volatile losses.

Table 3. Number of samples in which the HCB level increased or decreased after the culinary treatment. χ^2 test

EFFECT	GRILLING	ROASTING	COOKING	TOTAL
INCREASE	2	3	6	11
DECREASE	23	22	19	64
TOTAL	25	25	25	75
χ^2		p>0.05		

Van Renterghem (1976) reported a significant decrease of HCB residue level ($p<0.05$) in pasteurised milk samples (73°C/15 sec). Pre-sterilisation (137°C/10 sec) followed by a sterilisation at 118°C for 10 minutes produced a significant increase ($p<0.05$) of the HCB content. Pre-sterilisation followed by uperisation at 150°C for 2.4 sec. caused a more significant increase of the HCB content. The author considered that the heat treatment and homogenization occurring in both sterilised and uperised samples increase the extractability of physiologically incorporated organochlorine pesticides due to the affinity of these compounds with the lipoproteins of the fat globule membrane. On the other hand, Garrido Fernández (1990) made studies on milk samples spiked with 3 ppm of HCB. HCB content was reduced by 73.7% after sterilising at 115°C for 15 min without homogenising.

The current FAO/WHO specifications (1984, 85) recommend "absence" for the human daily intake of HCB, but the previous conditional value was 0.6 μg/kg body weight. Data available from the Meat Annual Report (1990) show that the individual ovine meat consumption in Spain in 1988 was 4.278 kg/person/year.

HCB mean contents after culinary treatments ranged from 0.039 to 0.041 μg HCB/g fat, which means an ADI of 0,09-0.10 μg HCB/day, representing 0.21-0.24% of the conditional value (FAO/OMS 1978). However, considering the highest HCB levels detected (from 0.172 to 0.202 μg HCB/g fat), which means an ADI of 0.40-0.47 μg HCB/day, nearly 1% of conditional value (FAO/OMS 1978) may be reached.

We conclude that any of the three treatments studied (cooking, roasting, grill) leads to a similar reduction of the HCB levels in chop, leg and lap samples, which is approximately 18%. Lamb meat contributes to the pesticide daily intake with 0.10 μg HCB/day. Even considering the highest HCB contents, our results are below the 1% of 42 μg/day (conditional value), the ADI previously suggested by FAO/WHO (1978).

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