Influence of Meat Processing and Meat Starter Microorganisms on the Degradation of Organochlorine Contaminants

Susana Bayarri, Antonio Herrera,* M. Pilar Conchello, Agustín A. Ariño, Regina Lázaro, and Cristina Yagüe

Department of Animal Production and Food Science, Veterinary Faculty, University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain

The influence of processing of different dry-cured sausages on the degradation of organochlorine pesticide residues such as hexachlorobenzene (HCB), α-, *β*-, *γ*-hexachlorocyclohexane (HCH), and *p*,*p*′-DDE was investigated. The residual contamination was analyzed at different stages of the sausage's ripening by capillary gas−liquid chromatography with electron capture detector. Furthermore, the degradative capability of the microorganisms used as starters in the manufacture of such meat products (*Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Micrococcus varians*) was studied in vitro. The *M. varians* strain was able to degrade some of the compounds tested. Significant reductions ($p < 0.05$ and $p < 0.01$) of HCB (12.7%) and *p*,*p*′-DDE (17.7%) were observed. However, the technological process of curing did not exert any significant effect on the residue levels ($p > 0.05$).

Keywords: *Organochlorines; meat products; processing; starter microorganisms; degradation*

INTRODUCTION

The widespread use of organochlorine pesticides (OCPs) for plant protection or animal hygiene has given notable positive results. However, as a result of the high stability of some of them, residues of these substances are found worldwide and often in the food chain. The organochlorine residues predominantly accumulate in the lipid fractions, by which animal fatty foods have become a major route of exposure for humans.

Due to their adverse effect on the environment, the use of OCPs has been limited in most developed countries, and pesticide residues in foods are being monitored to ensure that public health is not endangered by violative residue concentrations (FDA, 1991; Gunderson, 1995a,b; Gallo et al., 1996; Urieta et al., 1996; Lázaro et al., 1996). In this way, there is increasing interest of governments, research centers, and consumers in knowing which factors could contribute to reduce such contamination.

Previous research has demonstrated that food processing reduces organochlorine contamination, supplying foods less harmful to human health than raw materials. Thus, Conchello et al. (1993a,b) reported significant losses (18−39%) of *γ*-HCH (lindane) and HCB levels in meat after culinary treatments (grilling, roasting, or cooking). In a like manner, Kubacki and Lipowska (1980) and Ariño et al. (1993) observed a reduction of up to 28% of lindane content by commercial processing of dry-cured sausages. Shivankar and Kavadia (1992) found a 94−100% reduction in heptachlor and heptachlor epoxide in vegetables by washing, peeling, and cooking, and Jodral et al. (1995) observed that DDT and its metabolites were eliminated by 16−59% after pasteurization of milk. However, some treatments are ineffective due to the high stability of residues, as shown in the study of Bentabol et al. (1995), in which ripening of cheese did not cause any effect on HCB, aldrin, dieldrin, heptachlor, heptachlor epoxide, and DDT and its metabolites, or in the study of Ariño et al. (1995), in which no reduction of *p*,*p*′-DDE after curing of meat sausages was reported.

Additionally, it has also been demonstrated in vitro that microorganisms of technological interest in the food industry could degrade these residues, although only limited scientific information is available. Mirna and Coretti (1979) observed a reduction of up to 30% of lindane and DDT by the action of *Micrococcus varians* (isolated from a meat starter culture). Peric et al. (1980) and Spiric et al. (1981, 1983) reported a 28−59% reduction of α-HCH, *β*-HCH, lindane, DDT, and methoxychlor in nutritive liquid media after incubation with diverse micrococci strains isolated from fermented sausages.

The present study was carried out to estimate the influence of processing of dry-cured sausages (meat products of high fat content, the manufacture and organoleptic characteristics of which are achieved by microbial activity) in reducing residues of HCB, HCHs, and DDTs, frequently detected in foodstuffs. Another objective of this investigation was to conduct a preliminary screening on the ability of different curing microorganisms (from meat starter cultures) for OCP degradation using a recently developed technique (Bayarri et al., 1997). The scarce scientific information related with this subject has also motivated the present investigation.

MATERIALS AND METHODS

Reagents. High-purity acetone and *n*-hexane and petroleum ether, ethyl acetate, cyclohexane, and isooctane of pesticide grade were purchased from Lab-Scan. Each batch of solvents was subjected to a solvent purity test for residue analysis suitability, according to Association of Official Ana-

* Author to whom correspondence should be addressed.

S0021-8561(97)00932-1 CCC: $15.00
Published on Web 07/15/1998

Table 1. Compositions of the Meat Products Studied

meat product	description
chorizo vela	80% lean pork meat and 20% belly fat; salts, garlic, red pepper, starter cultures; filled into collagen casing of 80 mm diameter
chorizo de Pamplona	72% lean pork meat and 28% back fat; salts, garlic, red pepper, starter cultures; finely chopped and filled into collagen casing of 80 mm diameter
salchichón	60% lean pork meat and 40% belly fat; salts, garlic, black pepper, white pepper, starter cultures; filled into collagen casing of 85 mm diameter (50 mm for spiked salchichón)

lytical Chemists recommendations (AOAC, 1990), and no interfering impurities were observed.

Sodium sulfate (Na_2SO_4) anhydrous (Carlo Erba) was purified by heating in a furnace at 600 °C for 5 h.

Reference standards of the organochlorine pesticides were obtained from Supelco and Dr. Ehrenstorfer.

Meat starter cultures SAGA I (*Pediococcus acidilactici*) and SAGA L (*Pediococcus pentosaceus* and *Micrococcus varians* 1:1) were manufactured by QUEST International and supplied by Amerex Laboratories (Madrid, Spain).

Liquid culture media for the in vitro assays were the following: tryptic soy broth (TSB, Difco); De Man Rogosa & Sharpe medium (MRS, Merck); and mineral salts medium containing Na_2HPO_4 (7 g/L), KH_2PO_4 (3 g/L), NaCl (0.5 g/L), NH_4Cl (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.25 g/L), and yeast extract (Difco) (1 g/L).

Water was distilled using a MilliQ water purification system and extracted with *n*-hexane prior to preparation of the liquid media.

Sample Preparation. *Sausage Curing Experiments.* The influence of the technological process of curing on naturally incurred residues of HCB, α-, β-, and γ-HCH, and DDTs has been investigated in three different typical Spanish pork cured sausages known as "chorizo vela", "chorizo de Pamplona", and "salchichón", manufactured with a mixture of commercially available meat starter cultures, SAGA I and SAGA L.

A parallel experiment was carried out with samples of "salchichón", spiked with a standard mixture of HCB, HCHs, and *p*,*p*′-DDE at two different concentrations (200 and 1000 ng/g on a lipid basis, approximately). The standard mixture of organochlorine compounds in acetone was added dissolved in lard to the raw meat product, according to the method of Kubacki and Lipowska (1980). Raw material had been analyzed before spiking, and no residues were detected.

Five sausages of chorizo vela, five sausages of chorizo de Pamplona, and five sausages of salchichón were manufactured in the processing plant of Cárnicas KIKO S.A.L. (Los Sanfermines, Navarra), according to usual practices. Processing of spiked salchichón (six replicates with a level of contamination of ≈200 ng/g on a lipid basis and another six replicates with a level of contamination of ≈1000 ng/g on a lipid basis) was carried out in our laboratory in a heater with dispositive of control of temperature and relative humidity (RH). The samples were held for 48 h at 25 °C and 85−90% RH, allowing for fermentation, and ripened at 12−17 °C until the end of the process (19 days for chorizo vela, 33 days for chorizo de Pamplona, and 28 days for both salchichón batches). Table 1 describes the composition of each meat product studied.

The evolution of contamination was studied at different days of ripening. Each sample was analyzed just before casing (raw material), after fermentation (at day 2), in the middle of processing (day 11 for chorizo vela, day 19 for chorizo de Pamplona, day 10 for spiked salchichón, and day 15 for salchichón) and at the end of the process (day 21 for chorizo vela, day 35 for chorizo de Pamplona, and day 30 for both salchichón batches).

In Vitro Experiments with Starter Microorganisms. P. acidilactici was isolated from the commercially available meat starter culture SAGA I; *P. pentosaceus* and *M. varians* were isolated from the commercially available meat starter culture SAGA L. Isolation of microorganisms was achieved by differentiation of colonies in nutritive agar, microscopic cellular morphology, and the catalase test, according to *Bergey's Manual of Determinative Bacteriology* (1994). The strains were stored in skim milk vials at −18 °C until utilized.

The in vitro study was performed in triplicate in two different liquid media: a nutritive medium (De Man, Rogosa, and Sharpe for *P. acidilactici* and *P. pentosaceus*; tryptic soy broth for *M. varians*) and a mineral salts medium, acidified to pH 6.2 for lactic strains. A 18-h-old actively growing broth culture was used to inoculate the sterile media to give a concentration of 10^5 cells/mL.

Tubes containing 9.9 mL of the sterilized liquid medium inoculated with an individual microorganism (10^5 cells/mL) were spiked with 0.1 mL of a standard solution of HCB, HCHs, and *p*,*p*′-DDE in acetone, in which each compound was at 100 μg/mL. Noninoculated samples containing the organochlorine compounds were used as control. All tubes were incubated at 30 °C on a rotary shaker for 7 days.

Chemical Analysis. *Sausage Samples.* Fat and organochlorine residues were extracted with petroleum ether, as described in the *U.S. Pesticide Analytical Manual* (FDA, 1994), and fat content was determined according to the method of Lázaro et al. (1995). The extracts were redissolved in ethyl acetate/cyclohexane (1:1) at a concentration of 0.75 g of fat/5 mL and cleaned up by gel permeation chromatography (GPC) on an ABC SP-1000 apparatus with a Bio-Beads SX-3 column. The elution was carried out with ethyl acetate/cyclohexane (1:1) at the rate of 5 mL/min. The fat and other coextractants were eluted and discarded in the first 105 mL (21 min of dump time); the analytes were collected in a second fraction of 155 mL (31 min of collection time).

Chromatographic analyses were performed with a Hewlett-Packard HP 5890 gas chromatograph with ^{63}Ni electron capture detector (ECD), equipped with an automatic injector HP 7673A. Fused silica capillary columns coated with 5% phenyl methyl polisiloxane (007-2, 50 m × 0.25 mm i.d. × 0.25 μm film thickness) and cyanopropyl phenyl methyl polisiloxane (007-608, 30 m × 0.53 mm i.d. × 0.80 μm film thickness) were used for determination. Quantification was based on the external standard method using a multicalibration curve. Spiked samples were diluted prior to injection in GLC in order to analyze them on the linear portion of the ECD. Data acquisition and processing were performed on an HP Vectra 486/33U computer with Hewlett-Packard Chemstation software.

Recoveries of organochlorine pesticides according to this method were determined with 10 samples of corn oil spiked with isooctane solutions of the investigated compounds as recommended by the Association of Official Analytical Chemists (AOAC, 1990) and ranged from 80 to 110% in agreement with Food and Drug Administration recommendations (FDA, 1994).

A blank analysis was carried out for every set of 10 samples to check for GLC-ECD interferences. Detection and quantification limits were calculated according to the definitions of the Dutch Discussion Group for Residue Analysis (Haagsma et al., 1995) and the recommendations of the International Union of Pure and Applied Chemistry (IUPAC, 1978) and the Royal Society of Chemistry (Analytical Methods Committee, 1987). Table 2 lists the detection and quantification limits established for each compound and recoveries according to this method.

In Vitro Experiments. After incubation, the residues of OCPs remaining in the culture media were extracted with hexane following the method described by Bayarri et al. (1997). Quantification of biodegradation was carried out by capillary gas−liquid chromatography with the fused silica column coated with 5% phenyl methyl polysiloxane (007-2). The chromatograms of the extracts from the inoculated and noninoculated samples were compared to determine whether the microorganism had altered the residual amount of the organochlorine compounds in the medium.

Table 2. Recovery, Detection, and Quantification Limits of Each Pesticide According to the Analytical Technique

chemical	recovery ± RSD (%)	detection and quantification limit[a] (ng/g on lipid basis)
HCB	94.1 ± 10.2	1
α-HCH	95.7 ± 11.9	3
p,p′-DDE	102.7 ± 10.4	3
o,p′-DDE	88.5 ± 12.3	4
o,p′-DDD	94.6 ± 11.3	5
p,p′-DDD	91.8 ± 11.4	5
γ-HCH[b]	96.6 ± 12.1	5[c]−7[d]
o,p′-DDT	80.0 ± 15.1	10
β-HCH	83.0 ± 17.0	15
p,p′-DDT	85.8 ± 7.5	19

[a] When no interfering peaks were observed in the blanks, detection and quantification limits of the pesticides were set to the minimum quantity of each one that had a linear response in the ECD. [b] It was observed an interfering peak in the blanks at the same retention time of this compound. [c] Detection limit. [d] Quantification limit.

For every in vitro assay, a procedural blank consisting of all reagents and glassware used during analysis was run to check for interferences and cross-contamination. Measurement of pH of the media at initial stage and at the end of the incubation period was performed with a Crison pH-meter.

Quality Control of Data. The quality of analytical data was assured by participation in two intercomparison exercises for chlorobiphenyls and organochlorine pesticides organized by the SOAFD Marine Laboratory, Aberdeen, U.K., and sponsored by the EEC Community Bureau of Reference (BCR).

RESULTS AND DISCUSSION

Influence of Meat Processing on Organochlorine Residue Levels. Overall, raw material of meat products (day 0) was slightly contaminated with organochlorine pesticides. Residue levels were very low and made difficult the study of contamination's evolution on naturally contaminated samples.

The percentage of samples contaminated with residues of OCPs was 66.7%. Of the DDT metabolites analyzed (DDT, DDD, and DDE), only residues of *p,p′*-DDE were found above the detection limit (3 ng/g on a lipid basis) in 53.3% samples. Residues of HCB appeared in 33.3% samples. Of the HCHs investigated, α-HCH was most frequently detected (present in 26.7% samples), followed by γ-HCH (13.3% of positivity), whereas residues of β-HCH were not found above the detection limit. In raw material of salchichón (day 0), residues of α-HCH were detected in 80% of samples, followed by HCB (60%), γ-HCH (40%), and *p,p′*-DDE (40%). In chorizo vela at day 0, residues of HCB and *p,p′*-DDE were detected in 40 and 100% of samples, respectively. Finally, only residues of *p,p′*-DDE were found in 20% of chorizo de Pamplona samples before casing. Therefore, data for only these residues are reported in the tables.

Mean concentrations of pesticide residues at different days of ripening of meat products, and statistical analysis of data by Friedman's nonparametric test (Sachs, 1978; Calvo, 1987), are shown in Table 3. To facilitate statistical analysis, values of samples below the laboratory quantification limit were set equal to half of the quantification limit (Kushner, 1976). Analysis of the data by Friedman's test indicated that the concentration of all residues investigated was constant throughout the curing of pork sausages studied.

The pattern of contamination detected in this study is similar to that traditionally observed in pork meat products in Spain. Most authors have reported the presence of HCB, HCHs, and DDE in these foods (Sánchez-Pérez et al., 1982; Pozo et al., 1982; García-Regueiro et al., 1987; Herrera et al., 1994). Pesticide levels were well below the respective maximum residue limits set by current European regulations in meat and meat products (Directive 86/363/CEE, currently modified by Directive 96/33/CE). The maximum residue limit for DDT (*p,p′*- and *o,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD) and γ-HCH is 1000 ng/g on a lipid basis, for α-HCH and HCB, 200 ng/g on a lipid basis, and for β-HCH, 100 ng/g on lipid basis.

The low levels of organochlorine residues determined in the Spanish dry sausages of our study pose little or no threat at all to consumers. However, it is advisable to study the changes in residue levels during technological or culinary treatments of food products, because the presence of chlorinated residues is still real (people do not make sausages from domestic animals only; wildlife is often used, which may be contaminated with higher levels of organochlorines), and the possibility of accidental waste or fraudulent use of these compounds is a potential concern. The presence of organochlorines at high levels in meat represents not only a toxicological risk for consumers but also an injury to the quality of end meat products, as stated by Cuñat (1984). Thus, the experiment with spiked samples allowed us to determine the evolution of the residues of interest during processing of dry-cured sausages. Results of modification of HCB, HCHs, and *p,p′*-DDE residue levels during ripening of spiked salchichón are summarized in Table 4.

Mean levels of HCB remained fairly constant throughout the process, indicating no effect on this pesticide ($p > 0.05$ by Friedman's nonparametric test). Thus, the mean level of HCB was 882 ng/g on a lipid basis in raw material and 852 ng/g after the 30 day curing period and 158 ng/g at day 0 and 172 ng/g at day 30 in the other batch of sausages. α-, β-, and γ-HCH were also found to be resistant to degradation under the conditions of the sausage ripening process. Thus, the mean levels in raw material were 880, 770, and 896 ng/g on a lipid basis, respectively, and at the end of the process were 836, 719, and 862 ng/g on a lipid basis, respectively. A slight reduction was noted in their concentrations from those which were present in the raw material, a reduction that was not statistically significant ($p > 0.05$). The mean concentrations in the other batch of sausages at day 0 (159, 151, and 167 ng/g of α-, β-, and γ-HCH, respectively) remained almost unchanged after processing. The mean levels of DDE in raw material were 743 and 141 ng/g on a lipid basis and showed no variation through ripening (739 and 131 ng/g, respectively).

In contrast with our results, several authors have reported degradation of chlorinated pesticides by curing. Thus, Mirna and Coretti (1979) found a 23−24% reduction in lindane (γ-HCH) and DDT in meat sausages by curing and smoking. Similarly, Kubacki and Lipowska (1980) reported 15 and 20% reductions in *p,p′*-DDE and γ-HCH levels in pesticide-fortified model sausages for 6 days.

In our laboratory, Ariño et al. (1993) found a 22−28% ($p < 0.05$) reduction in the α-HCH and lindane levels in pork sausage after 30 days of curing. It was also observed that mean levels of HCB and *p,p′*-DDE remained almost unchanged during processing (Ariño et

Table 3. Effect of Meat Processing on Naturally Incurred Residues of HCB, HCHs, and DDE in Dry Sausages

	mean ± SD (ng/g on lipid basis)				
sausage curing	HCB	α-HCH	β-HCH	γ-HCH	*p,p′*-DDE
salchichón					
raw material	2.0 ± 1.9	5.1 ± 2.9	nd[a]	3.5 ± 0.0	2.5 ± 1.4
sausage at 2nd day	2.6 ± 3.6	7.4 ± 3.9		3.5 ± 0.0	2.2 ± 0.9
sausage at 15th day	2.2 ± 3.1	4.4 ± 2.8		3.5 ± 0.0	1.8 ± 0.7
sausage at 30th day	2.2 ± 3.5	5.3 ± 2.8		4.3 ± 1.8	2.4 ± 1.3
Friedman's nonparametric test			$p > 0.05$		
chorizo vela					
raw material	1.8 ± 1.9	nd	nd	nd	5.7 ± 2.2
sausage at 2nd day	1.6 ± 2.4				7.4 ± 1.6
sausage at 11th day	1.8 ± 2.8				5.9 ± 2.4
sausage at 21th day	1.0 ± 1.1				6.4 ± 2.5
Friedman's nonparametric test			$p > 0.05$		
chorizo de Pamplona					
raw material	nd	nd	nd	nd	1.9 ± 0.9
sausage at 2nd day					2.4 ± 1.3
sausage at 19th day					2.3 ± 1.1
sausage at 35th day					1.5 ± 0.0
Friedman's nonparametric test			$p > 0.05$		

[a] Not detected.

Table 4. Effect of Processing on HCB, HCHs, and *p,p′*-DDE in Spiked Salchichón

	mean + SD (ng/g on lipid basis)			
chemical	raw material	day 2	day 10	day 30
HCB				
A[a]	881.7 ± 92.5	834.5 ± 72.6	814.4 ± 112.4	852.3 ± 21.7
B[a]	158.0 ± 12.2	178.5 ± 8.3	170.7 ± 14.7	171.9 ± 15.3
Friedman's nonparametric test		$p > 0.05$		
α-HCH				
A	879.7 ± 95.8	846.5 ± 70.9	804.1 ± 107.7	836.4 ± 23.1
B	158.8 ± 7.9	181.4 ± 9.7	170.2 ± 17.1	172.3 ± 18.0
Friedman's nonparametric test		$p > 0.05$		
β-HCH				
A	770.2 ± 37.0	762.7 ± 138.3	760.4 ± 120.5	718.9 ± 67.9
B	150.9 ± 18.6	157.6 ± 18.0	137.9 ± 26.6	143.3 ± 19.2
Friedman's nonparametric test		$p > 0.05$		
γ-HCH				
A	895.7 ± 102.2	874.8 ± 81.1	838.6 ± 119.5	862.0 ± 27.4
B	166.6 ± 8.5	188.5 ± 11.2	176.3 ± 18.6	178.3 ± 16.1
Friedman's nonparametric test		$p > 0.05$		
p,p′-DDE				
A	743.0 ± 111.0	770.6 ± 94.1	701.7 ± 106.1	739.2 ± 44.7
B	140.9 ± 4.0	155.1 ± 10.4	149.3 ± 28.1	130.7 ± 18.9
Friedman's nonparametric test		$p > 0.05$		

[a] A, highly contaminated batch of sausages (spiked at 1000 ng/g on a lipid basis, approximately); B, slightly contaminated batch of sausages (spiked at 200 ng/g on a lipid basis, approximately).

al., 1992, 1995). The significant hexachlorocyclohexane decrease observed was related to the growth of lactic acid bacteria and other ripening microorganisms. This fact could not be demonstrated in our study, in which samples were subjected to fermentation at 25 °C for 48 h immediately after casing. This step has been reported to produce a dramatic pH decrease in Spanish pork sausage (Roncalés et al., 1991), and Mirna and Coretti (1979) reported inhibition of the pesticide degrading enzymatic system in vitro by a decrease in the pH value, which may explain the lack of significant pesticide reduction in our samples. Moreover, these researchers noted greater reductions of DDT and lindane levels after curing and smoking of sausages manufactured without microbial starter cultures than in those made with starters.

In other related research with dairy products, Smoczynski et al. (1974) also observed that residue levels did not vary after the processing of the soft cheeses Brie and Camembert, suggesting that the pesticides, due to the protective effect of the fat, became inaccessible to the ripening microorganisms.

Degradation Capability of Meat Starter Microorganisms. Table 5 summarizes the percentage of the investigated compounds remaining in culture media after incubation with each microorganism. This percentage is related to the concentration of chemical remaining in noninoculated media. Statistical analysis of data was carried out by Mann−Whitney's nonparametric test. Observed variations of ±10% in residue levels were not considered significant. These differences were most probably attributable to the analytical method.

P. pentosaceus and *P. acidilactici* did not show ability to degrade the tested contaminants in a nutrient medium. Overall, mean levels of OCPs remained almost unchanged after the incubation period. Similarly, no degradation of pesticides in the mineral salts medium was observed.

In the nutrient medium inoculated with *M. varians*, slight modifications of HCB, HCHs, and *p,p′*-DDE levels were observed. These variations were not statistically significant ($p > 0.05$), indicating no activity on these pesticides. However, it confirmed the ability of *M. varians* to degrade some of these highly persistent com-

Table 5. Residual Amounts Remaining in the Culture Media after Incubation with Different Microbial Starters

	percentage[a] ± SD				
microorganisms and media	HCB	α-HCH	β-HCH	γ-HCH	*p,p′*-DDE
P. pentosaceus					
nutritive medium	104.5 ± 2.9	101.9 ± 2.4	100.8 ± 2.0	100.1 ± 2.6	105.4 ± 1.8
mineral salts medium	101.2 ± 6.3	102.2 ± 6.0	102.0 ± 13.0	102.9 ± 5.7	106.3 ± 4.6
M. varians					
nutritive medium	97.9 ± 3.5	99.0 ± 4.6	108.9 ± 6.7	98.8 ± 4.8	94.1 ± 4.9
mineral salts medium	87.3 ± 8.2[b]	98.8 ± 1.8	106.9 ± 10.0	99.2 ± 1.9	82.3 ± 7.9[c]
P. acidilactici					
nutritive medium	103.0 ± 6.2	100.1 ± 7.8	98.9 ± 5.9	98.3 ± 7.1	104.5 ± 5.4
mineral salts medium	103.1 ± 1.5	103.5 ± 2.4	101.2 ± 9.4	102.6 ± 3.7	99.9 ± 8.6

[a] Related to the concentration of chemical remaining in noninoculated media. [b] Significantly decreased by 12.7% ($p < 0.05$ by Mann−Whitney's nonparametric test). [c] Significantly decreased by 17.7% ($p < 0.01$ by Mann−Whitney's nonparametric test).

pounds in the mineral salts medium. As Table 5 shows, the microorganism significantly reduced the levels of HCB and *p,p′*-DDE by 12.7 and 17.7%, respectively.

The presence of chlorine in a molecule contributes to a compound being stable, and this could be a reason of the inactivity of lactic acid bacteria (*P. pentosaceus* and *P. acidilactici*) in our study. The results of the in vitro study agree with those obtained after ripening of meat sausages inoculated with these two strains, as previously discussed.

Similar to our results, Mirna and Coretti (1979) did not observe any significant reduction of DDT or lindane in MRS medium after incubation with two strains of lactic bacteria belonging to the genus *Lactobacillus*. The inactivity of microorganisms was related to the low pH of the culture medium after the incubation period. In this sense, several authors labled the pH as a factor that influences the microbial degradation process (Furukawa, 1982; Fewson, 1988). In our experiment, the pH of the nutritive medium (MRS) decreased from 6.6 to ≈4.0 after incubation with lactic acid bacteria.

In contrast, *M. varians* did show capability to degrade some of the organochlorines, but only in the mineral salts medium. This fact is of great interest for the meat industry because it implies that the microorganism is capable of reducing the toxicological risk of highly stable compounds such as HCB or *p,p′*-DDE. However, in the presence of nutrients, such as liquid medium or meat sausages under the conditions of our study, the strain did not show any activity. The different degradation results obtained in the different media could be explainable because the microbial metabolic activity can be influenced by environmental circumstances such as the composition of culture media, as Boethling (1993) and Marshall and Law (1984) indicated.

Katayama and Matsumura (1993) pointed out the possibility that the microorganism showed preference for other organic substrates instead of the pesticides in the medium. In addition to nutrient levels, the lack of availability of the pollutant may be a limiting factor. The xenobiotic may remain bound to some components of the medium, avoiding the degradative activity of microorganisms, as observed by Langlois et al. (1970) with the pesticide heptachlor and milk casein.

In another in vitro study with different strains of micrococci isolated from fermented sausages, Peric et al. (1980) also demonstrated the capability of microorganisms to degrade diverse organochlorines, the reductions being higher than those obtained in our study (up to 59%).

Contrary to our results, Spiric et al. (1981, 1983) observed that diverse micrococci strains did degrade DDT and lindane in the presence of nutrients, and Mirna and Coretti (1979), in their in vitro study with a strain of *M. varians* isolated from a commercially available culture, observed a reduction of up to 30% of lindane and DDT in the liquid media tryptic soy broth (TSB). In agreement with the results obtained in our study with HCHs, several authors stated that the pesticide lindane and the diverse isomers of hexachlorocyclohexane are easily biodegradable in anaerobic conditions, being highly resistent to the action of aerobic microorganisms (Heritage and MacRae, 1977; Ohisa and Yamaguchi, 1978; Haider, 1979). In addition to this, β-HCH is highly persistent due to the spatial distribution of chlorines in its molecule (Haider et al., 1974). However, Sahu et al. (1990, 1992) did demonstrate that an aerobic microorganism (*Pseudomonas* sp.) was able to degrade α-, β-, δ-, and γ-HCH in aerobic conditions.

In summary, it can be inferred from our results that the process of curing of pesticide-spiked sausages did not exert a significant effect on the decrease of HCB, HCHs, or DDE levels. Nevertheless, it was observed that the in vitro degradative capability of one microbial strain used as starter in the manufacture of dry sausages (*M. varians*) may be applicable in the food industry. However, further study in this area is required before the feasibility of such a strategy can be determined.

ABBREVIATIONS USED

OCPs, organochlorine pesticides; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyltrichloroethylene; DDD, dichlorodiphenyldichloroethane; GLC-ECD, gas−liquid chromatography-electron capture detector; SD, standard deviation; RSD, relative standard deviation.

ACKNOWLEDGMENT

We express our gratitude to Cárnicas KIKO (Los Sanfermines, SAL) for its collaboration throughout this work and to Quest International and Amerex Laboratories for supplying the starter cultures. We thank Nieves Ortega for her assistance in the sample analysis.

LITERATURE CITED

Analytical Methods Committee. Recommendations for the definition, estimation and use of the detection limit. *Analyst* **1987**, *112*, 199−204.

Ariño, A.; Herrera, A.; Conchello, M. P.; Pérez, C. Hexachlorobenzene residues in Spanish meat products after cooking, curing and long-term ripening. *J. Food Prot.* **1992**, *55* (11), 920−923.

Ariño, A.; Herrera, A.; Conchello, M. P.; Lázaro, R.; Pérez, C. Hexachlorocyclohexane residues in meat products after processing. *J. Food Compos. Anal.* **1993**, *6*, 55−61.

Ariño, A.; Lázaro, R.; Conchello, P.; Bayarri, S.; Herrera, A. The effect of processing on incurred residues on DDE in meat products. *Food Addit. Contam.* **1995**, *12* (4), 559−566.

Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; AOAC: Arlington, VA, 1990.

Bayarri, S.; Conchello, P.; Ariño, A. A.; Lázaro, R.; Herrera, A. Evaluation of an analytical method for an *in vitro* study of degradation of organochlorine compounds by "meat starter" microorganisms. *Pestic. Sci.* **1997**, *50*, 120−126.

Bentabol, A.; Garrido, M. D.; Martínez, M. P.; Angulo, R.; Acosta, I.; Gallego, M. C.; Jodral, M. Evolución de pesticidas organoclorados en quesos de cabra madurados. I Congreso Internacional de Tecnología y Calidad Alimentaria. *Residuos Fitosanitarios en Alimentos*; La Rioja, Spain, 1995.

Bergey's Manual of Determinative Bacteriology, 9th ed.; Hensye, W. R., Ed.; Williams & Wilkins: Baltimore, MD, 1994.

Boethling, R. S. Biodegradation of xenobiotic chemicals. *Handbook of Hazardous Materials*; Academic Press: New York, 1993; pp 55−67.

Calvo, F. *Estadística Aplicada*; Deusto S.A.: 1987.

Conchello, Mª P.; Herrera, A.; Ariño, A.; Lázaro, R.; Bayarri, S. Effect of several kitchen treatments on hexachlorocyclohexane residues in ovine meat. *Bull. Environ. Contam. Toxicol.* **1993a**, *51*, 612−618.

Conchello, M×bb P.; Herrera, A.; Ariño, A.; Lázaro, R.; Pérez, C. Effect of grilling, roasting and cooking on the natural hexachlorobenzene content of ovine meat. *Bull. Environ. Contam. Toxicol.* **1993b**, *50*, 828−833.

Cuñat, P. Residuos de plaguicidas en productos cárnicos. *Rev. Agroquim. Tecnol. Aliment.* **1984**, *24* (3), 423−431.

FDA (Food and Drug Administration). Residues in foods, 1990. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 121A−141A.

FDA (Food and Drug Administration). *Pesticide Analytical Manual. Volume I. Methods which detect multiple residues*, 3rd ed.; U.S. Department of Health and Human Services: Washington, DC, 1994 (revised 1996).

Fewson, C. A. Biodegradation of xenobiotic and other persistent compounds: the causes of recalcitrance. *Trends Biotechnol.* **1988**, *6*, 148−153.

Furukawa, K. Microbial degradation of polychlorinated biphenyls. In *Biodegradation and detoxification of environmental pollutants*; Chakrabarty, F. L., Ed.; CRC Press: Boca Raton, FL, 1982.

Gallo, P.; Castellano, V.; Serpe, L. Monitoraggio dei residui di pesticidi organoclorurati ed organofosforati in alimenti di origine animale: resultati e considerazioni relativi agli anni 1990−1994. *Ind. Aliment.* **1996**, *35*, 253−257.

García-Regueiro, J. A.; Díaz, I.; Monfort, J. M. Determinación de plaguicidas organoclorados, mediante cromatografía de gases con columna capilar (CGC), en carne y productos cárnicos del mercado de Gerona [Determination of organochlorine pesticides by capillary column gas chromatography (CGC) in meat and meat products from markets in Gerona (Spain)]. *Rev. Agroquim. Tecnol. Aliment.* **1987**, *27* (3), 405−417.

Gunderson, E. L. Dietary intakes of pesticides, selected elements, and other chemicals: FDA total diet study, June 1984-April 1986. *J. AOAC Int.* **1995a**, *78* (4), 910−921.

Gunderson, E. L. FDA total diet study, July 1986-April 1991, dietary intakes of pesticides, selected elements, and other chemicals. *J. AOAC Int.* **1995b**, *78* (6), 1353−1363.

Haagsma, N.; Ellen, G.; Stephany, R. W.; De Ruig, W. G. Dutch Discussion Group for Residue Analysis (ORA): definitions for the determination of drug residues in food of animal origin. Final report, 1995.

Haider, K. Degradation and metabolization of lindane and other hexachlorocyclohexane isomers by anaerobic and aerobic soil microorganisms. *Z. Naturforsch. Sect. C* **1979**, *34*, 1066−1069.

Haider, K.; Jagnow, R.; Kohnen, R.; Lim, S. U. Degradation of chlorinated benzenes, phenols and cyclohexane derivatives by benzene and phenol utilizing soil bacteria under aerobic conditions. *Arch. Microbiol.* **1974**, *96*, 183−200.

Heritage, A. D.; MacRae, I. C. Degradation of lindane by cell-free preparations of *Clostridium sphenoides*. *Appl. Environ. Microbiol.* **1977**, *34*, 222−224.

Herrera, A.; Ariño, A.; Conchello, M×bb P.; Lázaro, R.; Bayarri, S.; Pérez, C. Organochlorine pesticide residues in Spanish meat products and meat of different species. *J. Food Prot.* **1994**, *57* (5), 441−444.

IUPAC (International Union of Pure and Applied Chemistry). Nomenclature, symbols, units and their usage in spectrochemical analysis−II. *Spectrochim. Acta* **1978**, Part B.

Jodral, M.; Martínez, P.; Angulo, R.; Gallego, M. C.; Pozo, R. El proceso de la pasterización y su influencia sobre el DDT y sus metabolitos. *Alimentaria* **1995**, *266*, 25−28.

Katayama, A.; Matsumura, F. Degradation of organochlorine pesticides, particularly endosulfan, by *Trichoderma harzianum*. *Environ. Toxicol. Chem.* **1993**, *12*, 1059−1065.

Kubacki, S. J.; Lipowska, T. The role of food procesing in decreasing pesticide contamination of foods. In *Food and Health: Science and Technology*; Birch, G. G., Parker, K. J., Eds.; Applied Science Publishers: London, 1980.

Kushner, E. J. On determining the statistical parameters for pollution concentration from a truncated data set. *Atmos. Environ.* **1976**, *10*, 975−979.

Langlois, B. E.; Collins, J. A.; Sides, K. G. Some factors affecting degradation of organochlorine pesticides by bacteria. *J. Dairy Sci.* **1970**, *53* (12), 1671−1675.

Lázaro, R.; Bayarri, S.; Conchello, P.; Ariño, A.; Herrera, A. Comparación de dos técnicas de extracción de materia grasa para la determinación de residuos organoclorados en alimentos (Comparison between two techniques for fat extraction from foods prior to the determination of chlorinated residues). *Grasas Aceites* **1995**, *46* (1), 35−38.

Lázaro, R.; Herrera, A.; Ariño, A.; Conchello, M. P.; Bayarri, S. Organochlorine pesticide residue in total diet samples from Aragon (Northeastern Spain). *J. Agric. Food Chem.* **1996**, *44* (9), 2742−2747.

Marshall, V. M. E.; Law, B. A. *Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk*; Davies, F. L., Law, B. A., Eds.; Elsevier Applied Science Publishers: Amsterdam, 1984.

Mirna, A.; Coretti, K. Influence of processing on the degradation of pesticides in meat products. *Meat Sci.* **1979**, *3*, 97−108.

Ohisa, M.; Yamaguchi, M. Gamma-BHC degradation accompanied by the growth of *Clostridium* isolated from paddy field. *Agric. Biol. Chem.* **1978**, *42*, 1819−1823.

Peric, M.; Raseta, J.; Visacki, M. S.; Spiric, A. Degradation of organochlorine pesticides as influenced by micrococci isolated from fermented sausages. *Technol. Mesa* **1980**, *21* (5), 132−133.

Pozo, R.; Polo, L. M.; Jodral, M.; Herrera, A. Investigaciones sobre el fungicida hexaclorobenceno y otros pesticidas organoclorados en productos cárnicos enlatados de cerdo (Studies on the fungicide hexachlorobenzene and other organochlorine pesticides in canned pork products). *Arch. Zootecn.* **1982**, *31* (121), 281−291.

Roncalés, P.; Aguilera, M.; Beltran, J. A.; Jaime, I.; Peiro, J. M. The effect of natural and artificial casing on the ripening and sensory quality of a mold-covered dry sausage. *Int. J. Food Sci. Technol.* **1991**, *26*, 83−89.

Sachs, L. *Estadística Aplicada*, 1st ed.; Labor ed.; Barcelona, Spain, 1978.

Sahu, S. K.; Patnaik, K. K.; Sharmila, M.; Sethunathan, N. Degradation of α-, β-, and γ-hexachlorocyclohexane by a soil bacterium under aerobic conditions. *Appl. Environ. Microbiol.* **1990**, *56* (11), 3620−3622.

Sahu, S. K.; Patnaik, K. K.; Sethunathan, N. Dehydrochlorination of δ-isomer of hexachlorociclohexane by a soil bacterium *Pseudomonas* sp. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 265−268.

Sánchez-Pérez, A.; Casado, L.; Jiménez, R. Niveles de residuos de plaguicidas organoclorados en grasas animales y alimentos ricos en materia grasa. *Bol. CeNAN* **1982**, *11/12*, 3−4.

Shivankar, V. J.; Kavadia, V. S. Effect of post-harvest processes in heptachlor residues in potato, sweet potato and onion. *Trop. Agric. (Trinidad)* **1992**, *69* (1), 25−28.

Smoczynski, S.; Jaworski, J.; Borejszo, Z.; Kleppin, R. Clorowane weglowodory w serach dojrzewajacych. II. Romadur, Brie i Camembert. *Roczniki Inst. Przem. Mlecz.* **1974**, *2* (49), 55−60.

Spiric, A.; Visacki, V.; Raseta, J.; Peric, M.; Rajkovic, N. Kinetics of descomposition of DDT in meat products under the action of selected micrococci. *Technol. Mesa* **1981**, *22* (12), 344−345.

Spiric, A.; Visacki, V.; Raseta, J.; Peric, M.; Rajkovic, N. Kinetics of lindane degradation as influenced by selected strains of micrococci. *Int. Congr. Food Sci. Technol., 6th* **1983**, *2*, 70−71.

Urieta, I.; Jalón, M.; Eguileor, I. Food surveillance in the Basque Country (Spain). II. Estimation of the dietary intake of organochlorine pesticides, heavy metals, arsenic, aflatoxin M_1, iron and zinc through the Total Diet Study, 1990/91. *Food Addit. Contam.* **1996**, *13* (1), 29−52.

Received for review November 3, 1997. Revised manuscript received April 13, 1998. Accepted May 20, 1998. We thank the Diputación General de Aragón (DGA) for giving a 4-year scholarship to S.B. and the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for its financial support in the research projects ALI 95-0438 and ALI 96-0713. The present study has been awarded by the University of Zaragoza (Spain). Results concerning the *M. varians* experiment were presented to the 1st European Pesticide Residues Workshop "Pesticides in Food and Drink" held at Alkmaar, The Netherlands, June 10−12, 1996.

JF970932V